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Analyse des effets des rayonnements ionisants sur le système nerveux central du poisson zèbre : de la molécule à l'individu

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HAL Id: tel-04409706

<https://irsn.hal.science/tel-04409706>

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THÈSE DE DOCTORAT

Soutenue à Aix-Marseille Université
le 10 janvier 2023 par

Elsa Cantabella

Analyse des effets des rayonnements ionisants sur le système nerveux central du poisson zèbre : de la molécule à l'individu

Discipline

Sciences de l'Environnement

Spécialité

Environnement et Santé

École doctorale

ED 251 – Sciences de l'Environnement

Laboratoire/Partenaires de recherche

IRSET



Composition du jury

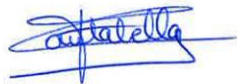
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Affidavit

I, undersigned, Elsa Cantabella, hereby declare that the work presented in this manuscript is my own work, carried out under the scientific direction of Christelle Adam-Guillermin and Thierry Charlier, in accordance with the principles of honesty, integrity and responsibility inherent to the research mission. The research work and the writing of this manuscript have been carried out in compliance with both the French national charter for Research Integrity and the Aix-Marseille University charter on the fight against plagiarism.

This work has not been submitted previously either in this country or in another country in the same or in a similar version to any other examination body.

Place Paris, date 21 octobre 2022



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Liste de publications et participation aux conférences

1) Liste des publications¹ réalisées dans le cadre du projet de thèse :

1. **Cantabella, E.**, Camilleri, V., Cavalie, I., Dubourg, N., Gagnaire, B., Charlier, T. D., ... & Armant, O. (2022). Revealing the Increased Stress Response Behavior through Transcriptomic Analysis of Adult Zebrafish Brain after Chronic Low to Moderate Dose Rates of Ionizing Radiation. *Cancers*, 14(15), 3793.
2. En cours: **Cantabella, E.**, Cavalie, I., Dubourg, N., Armant O. Reactive neurogenesis is induced after chronic exposure to ionizing radiation at moderate dose rates in adult zebrafish

2) Participation aux conférences² et écoles d'été au cours de la période de thèse :

1. International Congress of Toxicology – Maastricht, (2022)
2. Congrès des Femmes en sciences – Paris – Stand IRSN (2022)
3. Young Researchers in Life Science – Flashtalk (2021)
4. EcoBIM – EcotoQ – Présentation orale 2021
5. Congrès de l'Université (ED251) – Présentation orale (2021)
6. Journée des thèses IRSN – Poster (2019) & Oral (2021)

¹ Cette liste comprend les articles publiés, les articles soumis à publication et les articles en préparation ainsi que les livres, chapitres de livre et/ou toutes formes de valorisation des résultats des travaux propres à la discipline du projet de thèse. La référence aux publications doit suivre les règles standards de bibliographie et doit être conforme à la charte des publications d'AMU.

² Le terme « conférence » est générique. Il désigne à la fois « conférence », « congrès », « workshop », « colloques », « rencontres nationales et/ou internationales » ... etc.

Indiquer si vous avez fait une présentation orale ou sous forme de poster.

Résumé

A l'échelle des populations sauvages, des études en situation post-accidentelles ont montré une diminution du volume crânien d'oiseaux (Tchernobyl) et de fœtus de macaques (Fukushima) dans les territoires contaminés. D'autre part, des études de laboratoire montrent qu'une exposition néonatale de rongeurs aux RI réduit le nombre de progéniteurs neuronaux et conduit à des défauts cognitifs (i.e. apprentissage et mémoire). De récentes études attestent de l'apparition d'effets neurologiques chez l'adulte tels qu'une augmentation du risque d'apparition de la maladie de Parkinson ou des défauts cérébrovasculaires associés à une exposition avec des faibles débits de dose. Une altération de l'intégrité du système nerveux central lors d'une exposition chronique à faibles doses de RI serait donc susceptible d'affecter des processus majeurs comme la survie, la reproduction et le comportement.

Afin d'évaluer l'impact des radiations sur le cerveau du poisson zèbre adulte, trois débits de doses ($0,05 \text{ mGy.h}^{-1}$; $0,5 \text{ mGy.h}^{-1}$ et 5 mGy.h^{-1}) ont été retenus et maintenus durant 36 jours.

Grâce à des études transcriptomiques complétées d'hybridations d'ARN *in situ*, nous avons observé l'augmentation de l'expression de gènes impliqués dans la neurotransmission (à l'exception du système acétylcholinergique) ou dans l'axe du stress (cortisol en particulier) et identifié de potentiels biomarqueurs (ocytocine, arginine-vasopressine, cone rod homeobox) spécifiques d'une réponse aux expositions des rayonnements ionisants en comparaison avec la blessure physique du stabwound. A contrario des fortes doses de RI (diminution de neurogenèse) et similairement au stabwound, l'induction de la neurogenèse dans nos conditions expérimentales suggère la mise en place d'une compensation. Cette compensation est indépendante d'un processus inflammatoire et agirait sous les actions antioxydantes de l'ocytocine et de la mélatonine ou l'action anti-inflammatoire du cortisol et permettrait l'induction d'une neurogenèse réactive ainsi que l'expression de gènes impliqués dans la photoréception et présents au niveau du télencéphale.

Ces modifications à l'échelle moléculaire ont entraîné des perturbations comportementales, en particulier l'initiation d'un stress et la diminution de la réponse sociale spécifiquement chez les femelles poisson zèbres. Par conséquent, ces travaux

soulignent l'interaction complexe entre neurohormones, neurotransmetteurs et processus de neurogenèse afin de compenser les perturbations causées et modifiant l'homéostasie cérébrale suite à l'exposition chronique à des doses faibles à modérées de rayonnements ionisants.

Abstract

Nuclear accidents such as Fukushima and Chernobyl released high levels of IR in the environment, and studies reported the occurrence of microcephaly in yearling birds and *Macaca fuscata* fetuses. Laboratory studies demonstrated that neonatal exposure to IR reduces the number of neuronal progenitors and induces cognitive impairment (i.e., learning and memory). Emerging evidence describe neurological effects on adult exposure to radiation at dose rates much lower than previously thought in humans including elevated risk of Parkinson's disease and cerebrovascular defects. The alteration of central nervous system integrity caused by chronic exposure to low to moderate dose rates of IR could thus affect major processes such as survival, reproduction and behavior.

To evaluate the impact of IR on adult brain zebrafish, we exposed the whole organisms to three dose rates (0,05 mGy.h⁻¹; 0,5 mGy.h⁻¹ et 5 mGy.h⁻¹) for 36 days.

Transcriptomic studies associated with RNA *in situ* hybridization analysis showed the increased expression of genes with function in neurotransmission (except for acetylcholine system) or stress (cortisol specifically) and identified potential biomarkers (oxytocin, arginin-vasopressin, cone rod homeobox) specific to ionizing radiation exposure compared to physical injury through stabwound. In our experimental conditions, we observed the induction of neurogenesis contrarily to the impact of acute and high doses of IR where neurogenesis is reduced. While after stabwound injury, reactive neurogenesis is dependent on inflammatory processes, here neuro-inflammation was not initiated thus proposing a compensative system through potential anti-oxidative action of oxytocin and melatonin and the anti-inflammatory role of cortisol still allowing proper reactive neurogenesis and including the induction of genes involved in photoreceptor role in the telencephalon. These modifications on the molecular scale impacted the behavioral response specifically on female zebrafish with increased stress and reduction of sociability. Altogether, these findings highlight the intricate interaction between neurohormones, neurotransmission and neurogenesis process to balance the disturbed brain homeostasis caused by chronic exposure to low to moderate dose rates of IR.

Remerciements

Je souhaite tout d'abord remercier l'IRSN, et en particulier le SRTE sous la direction de François Paquet puis récemment de Rodolphe Gilbin pour le financement de ces 4 années de doctorat. Mes sincères remerciements sont également adressés aux membres du jury qui ont accepté d'évaluer ce travail de thèse. Encore merci à toutes les personnes m'ayant aidé durant ce projet de près ou de loin. En premier lieu, merci à mon encadrant Olivier Armant et mes directeurs de thèse Christelle Adam-Guillermin et Thierry Charlier. Olivier, merci pour ton accompagnement et ta confiance. Ce projet à tes côtés m'a fait évoluer tant scientifiquement que personnellement. Christelle, merci pour tes précieux conseils toujours très pertinents et nos agréables conversations au laboratoire lors de nos manip respectives. Thierry, merci pour ton superbe accueil à Rennes, toutes nos discussions et ton amour pour la science. Je tiens à remercier l'ensemble de l'équipe LECO. Un grand merci à Patrick, tous ces déjeuners à tes côtés pour discuter de science ou de bateau, pour la confiance que tu accordes aux doctorants et la bonne humeur que tu apportes au quotidien. Merci à Magali Coquillat pour ta bonne humeur et ta faculté à nous simplifier le quotidien. Merci à Isabelle et Virginie d'avoir partagé mon quotidien que vous avez égayé, pour toutes ces discussions lors des mes nombreuses hybridation *in situ*, pour ces mots croisés, pour les grands moments de rigolade. Merci Jean-François pour ta disponibilité et ta capacité à solutionner tout en un rien de temps ! Merci Nicolas pour ces pauses partagées mais particulièrement pour la salle comportement, ce fut un plaisir de travailler à tes côtés. Je souhaite aussi remercier l'ensemble des personnes auprès desquelles j'ai pu collaborer en dehors de l'équipe du LECO. Merci aux membres du comité de suivi de thèse, Xavier Cousin pour ton accueil à la Rochelle, et André Gilles pour tes conseils ainsi que votre suivi durant mon projet. Un grand merci à Christian Godon d'avoir consacré du temps pour m'enseigner les techniques du confocal. Je souhaiterais aussi remercier des personnes m'ayant accompagnée durant ma formation. Ainsi, merci à Saadia Kerdine-Römer de m'avoir introduite à la toxicologie, merci à François Coudoré qui m'a conseillé de me tourner vers l'IRSN pour mon stage de Master et merci à Maâmar Souidi qui m'a fait adorer la recherche. Je tiens également à remercier mes collègues thésards qui sont désormais mes amis. Loïc et ton amour pour la SF que tu as su me transmettre (promis ma prochaine lecture sera Azimov), ton humour et en particulier

celui de ton retour des vacances et pour ton accueil à bras ouverts dans votre bureau 😊.

Elizabeth, je ne vais pas m'étaler ici car la liste est longue et tu sais déjà tout ce que pourquoi je te remercie (escalade, théâtre, restaurant, your sweethome, nos folies, et surtout le cheval...ou pas !) cette rencontre n'est que le début d'une très belle aventure qui va nous régaler ! Clément, merci pour ton amour pour la nature, ton ironie parfois incomprise de ma part et ces moments de théâtre partagés ensemble. Magali pour toutes ces bières partagées à Manosque, nos séries débutées mais non terminées et nos balades à vélo ! Adrien pour toutes tes blagues et en particulier le fou rire dans le parc à Aix, c'est un souvenir ancré dans ma mémoire. Elio, pour la multitude d'histoires rocambolesques qui m'ont faites beaucoup rire ! Pour ta capacité limpide à connaître mon humeur et t'y adapter et pour tous ces mots appris, moi trop âgée pour les connaître. Je souhaite aussi remercier mes amis proches qui m'ont accompagné durant ces années. Merci Léo, mon support thésard et d'avoir insisté pour se voir même quand je me sentais submergée par la thèse, notre relation n'a fait que grandir 😊. Merci à BLEP de croire en moi et de m'avoir soutenue malgré la distance, ne changez rien you are my soul. Merci à tous les meudos (la liste est longue), mais quel plaisir de vous revoir à chaque fois, toujours pour des bons moments à partager. Merci aux coquillettes (Léa, Charlotte et Charlotte, Pauline, Lucie) en particulier nos moments à Ménerbes ancrés dans mon cœur. Merci à Valentine, tout d'abord pour cet emménagement rempli de folies et notre relation qui malgré la distance perdure et perdurera. Merci à Ophélie, même si on ne se voit qu'une fois tous les deux ans, ces fous rires ne font que renforcer notre amitié. Merci à Claire, amie de longue date qui m'accueille toujours à bras ouverts. Merci à Lucas, pour ton soutien infailible et ta capacité à m'apaiser dans mes plus grands moments de stress et de doute, tu as été un vrai pilier durant ce projet, et je remercie les concessions que cela a entraîné. Merci à la famille de Lucas, de votre volonté à comprendre ce projet et du soutien que vous m'avez apporté. Gracias a toda mi familia, mi tia, mis primos, mi yaya, mis hermanos y sobre todo a ti mama de todo el amor que me das, de la paciencia que tienes conmigo y de siempre apoyar mis sueños. Gracias a ti papa, aunque no puedas estar el d-day, se lo orgulloso que estarias de mi. Enfin je souhaite remercier toutes les personnes qui un jour ont pu m'accompagner ou m'aider d'une quelconque façon et m'amener à ce beau projet et vous souhaite le meilleur. Je souhaite terminer ces remerciements par une citation de Philippe Pollet-Villard qui m'inspire « Dans un voyage ce n'est pas la destination qui compte mais toujours le chemin parcouru, et les détours surtout. ». Bonne lecture !

Table of contents

Affidavit	3
Liste de publications et participation aux conférences	5
Résumé	7
Abstract	9
Remerciements	11
Table of contents	13
Common abbreviations list	15
Introduction	17
1. State of the art	21
1.1. Understanding radioactivity	21
1.1.1. The need of radioprotection due to human activities	21
1.1.2. Ionizing radiation: Definitions and biological effects	26
1.2. The central nervous system of vertebrates and model of choice: <i>Danio rerio</i>	29
1.2.1. Characteristics of <i>Danio rerio</i>	29
1.2.2. Constitutive neurogenesis	31
1.2.3. Regenerative neurogenesis	42
1.2.4. Ionizing radiation and the central nervous system	48
1.3. Social and Stress behaviors	50
1.3.1. The social response	50
1.3.2. The stress response	57
1.3.3. A possible crossroad between social and stress responses: the habenula	61
2. Research axes and methodology	63
2.1. Research axes	63
2.1.1. Axis I: From molecular effects to stress behavior and reduced sociability	63
2.1.2. Axis II: Common and differential features of IR response compared to regenerative neurogenesis induced by neuroinflammation	64
2.2. Global experimental design	65
3. Results & Discussion	67
3.1. Axis I: From molecular effects to stress behavior and reduced sociability	67
3.1.1. Objectives of scientific paper	67
3.1.2. Revealing the increased stress response behavior through transcriptomic analysis of adult zebrafish brain after chronic low to moderate dose rates of ionizing radiation	67
3.1.3. Main results and discussion	92
3.2. Axis II: Common and differential features of IR response compared to regenerative neurogenesis induced by neuroinflammation	96
3.2.1. Objectives of scientific paper	96

3.2.2. Reactive neurogenesis is induced after chronic exposure to ionizing radiation at moderate dose rates in adult zebrafish	96
3.2.3. Mains results & discussion	127
4. General conclusion and perspectives	132
REFERENCES	139
List of FIGURES	159
List of TABLES	161
ANNEX	162
	163

Common abbreviations list

ACTH: Adrenocorticotrophic hormone

Ascl: Achaete-scute like

ASN: Authority of Nuclear Safety) ensures nuclear safety and radioprotection.

BLBP: brain lipid-binding protein

BMP: Bone morphogenetic protein

CNS: Central nervous system

CRF: Corticotropin releasing factor/ equivalent to CRH: corticotropin releasing hormone

Cs137: Cesium 137

Cxcr: Chemokine (CXC motif) receptor

Dc: Central zone of the dorsal telencephalon

Dd: Dorsal zone of the dorsal telencephalon

DG: Dentate gyrus

DHb: Dorsal habenula

DI: Lateral zone of the dorsal telencephalon

Dm: Dorsomedial zone of the dorsal telencephalon

Dp: Posterior zone of the dorsal telencephalon

FGF: Fibroblast growth factor

GFAP: Glial fibrillary acidic

Gy: Gray

5-HT: 5-hydrotryptamine (serotonin)

Her: Human Epidermal Growth Factor Receptor-2

Hes: Hairy and enhancer of split-1

HPI: Hypothalamic pituitary interrenal

Hpl: Hours post lesion

IAEA: International Atomic Energy Agency

ICRP: International Commission on Radiological Protection

Id1: Inhibitor of DNA binding 1

INM: Interkinetic nuclear migration
IPN: Interpeduncular nucleus
IR: Ionizing radiation
IRSN: Institute for Radioprotection and Nuclear Safety
LET: Linear energy transfer
NEC: Neuroepithelial cells
Neurod1: neuronal differentiation 1
NSC: Neural stem cells
OPCs: Oligodendrocyte progenitor cells
OXT: Oxytocin
OXTR: Oxytocin receptor
PCNA: Proliferating cell nuclear antigen
POA: Preoptic area
PSA-NCAM: Polysialylated-neural cell adhesion molecule
RGC: Radial glial cells
RMS: Rostral migratory stream
ROS: Reactive oxygen species
SBN: Social behavior network
SDMN: Social decision-making network
SGZ: Subgranular zone
SVZ: Subventricular zone
Vd: Dorsal nucleus of the ventral telencephalon
Vp: Postcommissural nucleus of the ventral telencephalon
Vs: Supracommissural part of the ventral pallium
Vv: Ventral nucleus of the ventral telencephalo

Introduction

Initially, radioprotection aimed at caring protection of human populations without considering radiological risks on ecosystems. Indeed, first norms of International Commission on Radiological Protection (ICRP) in the seventies stand that human being the most radiosensitive species, a radioprotection focused on human will therefore protect all types of species (ICRP, 1977). However, ethical considerations on protecting biodiversity and wildlife, as well as concerned on the sustainability of ecosystemic services were raised in the following decades (ICRP, 1991; Pentreath, 1998; Whicker and Bedford, 1995). Several international agencies (International Atomic Energy Agency/IAEA, International Commission for Radiological Protection/ICRP, United Nations Scientific Committee on the Effects of Atomic Radiation/UNSCEAR) aimed at developing regulatory frameworks, recommendations, and safety concepts to provide handbooks to guide the evaluation for environmental protection against ionizing radiation and define requirements for ecological protection. Herein, in 2008, the UNSCEAR proposed that a threshold below 100 and 400 $\mu\text{Gy/h}$ of dose rates does not represent significant danger for terrestrial and aquatic populations respectively (UNSCEAR, 2008). Later, the development of European scientific projects (FASSET, ERICA and PROTECT) allowed the establishment of new reference values. Scientists developed a database (FREDERICA) to gather data from literature on the effect of radiotoxicants on mortality, reproduction, mutations and morbidity on sixteen taxonomic groups (Coppstone et al., 2008). This work led to the assessment of dose-response relationships for each species and the derivation of a recommended safety threshold at 10 $\mu\text{Gy}\cdot\text{h}^{-1}$ to protect 95% of the species with less than 10% of effects (EDR10) (Garnier-Laplace et al., 2010).

Several sources can contribute to the release of radiations into the environment. Beside natural sources (cosmic, telluric, water, atmospheric radioactivity) that can contribute to the background exposure to ionizing radiation (IR), artificial sources increase the risk of exposure of populations as well as the release in the environment (medical, industrial and military activity). In that context, releases from nuclear industry are regulated (in France) but can be subjected to accidental releases due to human mistakes or environmental catastrophes. The best but unfortunate illustrations are the

Chernobyl (1986) and Fukushima Daiichi (2011) nuclear accidents. In comparison to Chernobyl where the dose rates after 20 days reached 20000 mGy/day (UNSCEAR, 2008), in France, exposure to aquatic organisms by controlled releases of nuclear plants is around 0.01 mGy.year⁻¹ (Antonelli et al., 2021). Despite the radioactive decay of radionuclides, radiations are persistent in nuclear power plant accident sites with Cesium 137 (gamma radiation), Strontium 90 (beta radiation) or Plutonium 239 (alpha radiation) presence, consequently inducing a chronic exposure of wildlife still today in Chernobyl (0.0024 mGy/day to 24 mGy.day⁻¹) or Fukushima (2600 mGy.day⁻¹ in benthic biota 30 days after the accident) (Garnier-Laplace, 2013; Garnier-Laplace et al., 2011). Indeed, in contrast to human population that relocate, wildlife and plants do not and are thus continuously exposed to radionuclides. Moreover, many studies on rodents aimed at elucidating the effects of acute and high doses of radiations which mainly illustrates exposures in the medical field. In that context, it is crucial to study and understand the adverse outcomes of chronic low to moderate dose rates of ionizing radiation in order to protect the environment. Such data are not only important for danger characterisation on wild animals, but also to preserve in the long term the functioning of intact ecosystems for the next generations.

In ecotoxicology, the main parameters evaluated are resumed to reproduction, growth or survival without considering other individual aspects such as behavioral factors and effects at the molecular scale. The study of molecular events is however of great interest and needs a particular attention. Indeed, these effects occur earlier than the outcomes at the individual levels (mortality, reproduction or growth) and can thus be used as early warnings of detrimental effects observed at higher biological scales. Consequently, the study of the molecular mechanisms induced by exposure to ionizing radiation may bring to light the potentiality of a biomarker specific to chronic low to moderate dose rates of ionizing radiation. With the onset of toxicogenomic era, the limited predictivity of few biomarkers is compensated by the congruence of massive dataset that characterize the disturbance of biological pathways as a whole. At the organism scale, behavior is another integrative parameter that can predict effect on reproduction, growth or survival. For example, it was suggested that stress behavior or defect in social responses can impair foraging efficacy, defense against predator or mating choice in fish (Brown et al., 2003). The developing nervous system being one of the most sensitive organs to IR with the reproductive system, perturbation of its function with consequences on behavior is thus of major interest.

In that perspective, this thesis' work aims at deciphering the molecular effects of chronic low to moderate dose rates of ionizing radiation in the brain of adult zebrafish and how these events can affect behavior and particularly, social and stress responses. Thus, two axes articulate this study:

- Axis I: from molecular effects to stress behavior and reduced sociability
- Axis II: Common and differential features of IR response compared to regenerative neurogenesis induced by neuroinflammation

To evaluate the radiation induced impairment of brain adult zebrafish, three dose rates (0.05 mGy.h⁻¹, 0.5 mGy.h⁻¹, 5 mGy.h⁻¹) were chosen from the fact that exposure levels correspond to the domain of the low dose rates in humans (<6 mGy.h⁻¹) (UNSCEAR, 2010), are near the reference value for ecosystem radioprotection (0.01 mGy.h⁻¹) (Andersson et al., 2009) and are near the range of the dose estimated in fish after the Chernobyl accident (0.4 mGy.h⁻¹) (Lerebours et al., 2018).

4 parts constitute this work:

- The first part is the current general introduction
- The second part summarizes the actual knowledge needed to apprehend the objectives and results of the work. It presents (i) elementary notions on radioactivity through its history and the biological effects of ionizing radiation, (ii) the basic knowledge upon constitutive and regenerative neurogenesis with a focus on the radiological effects on brain and behavior, (iii) generalities on social and stress behavioral responses with emphasis on the zebrafish.
- The third part aims at developing the objectives of this thesis and the global experimental design used for this study.
- The fourth part deals with the presentation of the obtained results
- The fifth part corresponds to the general discussion and perspectives

1. State of the art

1.1. Understanding radioactivity

1.1.1. The need of radioprotection due to human activities

- **Röntgen and X-rays**

In the nineteenth century, the discovery of radioactivity transformed diverse fields of research, including physics and chemistry. Researchers developed and improved various devices using theories from multiple domains including physics and electromagnetism. At the end of 1895, Wilhelm Röntgen, professor of physics in Germany, discovered X rays, called from the unknown “x” in mathematics. This finding will revolutionize medicine by providing a direct insight in living organisms without the need of vivisection. He employed a modified version of Crook’s tube (**Figure 1**), originally used for the study of cathode rays. In this device cathode rays are created by the generation of electrons from a cathode and accelerated towards the anode in a sealed glass tube with partial vacuum.

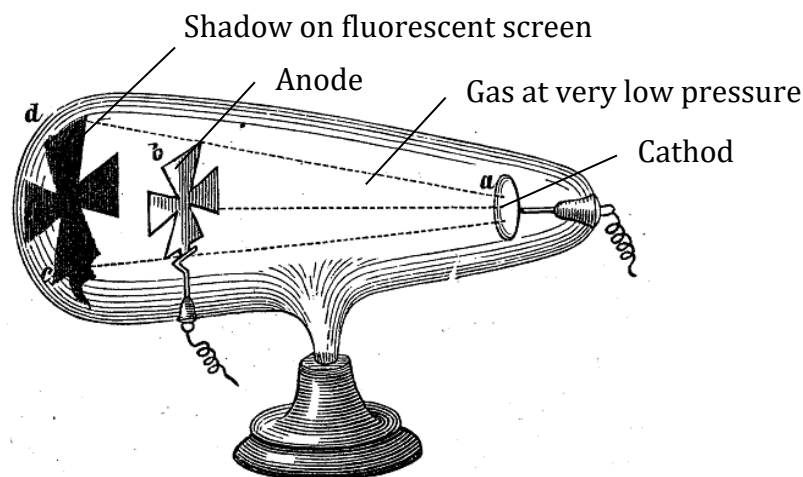


Figure 1 – Cathode tube ray. Electrons travel from the cathode towards the anode. The electrons are carried by their momentum to the glass tube behind the anode and emit light by falling back to their original energy level. The cross shaped anode in this tube is designed to cast a shadow. Adapted from Malley, 2011.

Röntgen covered the tube with a thick black cardboard, made the room completely dark, and surprisingly, a screen covered with barium platinocyanide (emitting fluorescence when hit by electrons) that was disposed away from the apparatus to prevent the escape of light, became illuminated. These rays could penetrate through its apparatus, travel far in air and their path was not modified by magnets. He proved his stunning discovery by photographing the human bones of his wife's hand (**Figure 2**). This discovery revolutionized medicine as physicians could now visualize fractures in their patients and predict the localization of tumours (Malley, 2011; Tubiana, 1996).



Figure 2 – First X-ray taken by Wilhelm Röntgen. From AncientPages.com, 2017.

- **Henri Becquerel and Uranium**

Henri Becquerel was issued from a family of scientists. He became in 1892 the director of Paris' Museum of Natural History where he oversaw a large collection of luminescent minerals that his father has gathered over the years. His particular interest was the luminescent uranium mineral, a heavy metal found in central European mines and commonly used for fluorescent tableware as glasses (**Figure 3**).



Figure 3 – Uranium glass under visible light (left) and under ultraviolet illumination (right). From “Uranium glass fluorescing – 11725688 | Science Photo Library,” n.d.

One year after Rontgen discovery, Becquerel wonders if the properties of phosphorescence and fluorescence of uranium salt are the same kind as X rays. Thus, he exposed uranium salts to the sun and as for X-rays, the paper photo reacted. However, the following days were cloudy and rainy so he could not experiment again and let a virgin photo paper with uranium salts in a drawer (**Figure 4**). Surprisingly, few days later, the paper photo reacted again and consequently uranium salts do not need to absorb light to glow. He thought that uranium had a property of hyper-phosphorescence because even after a long time, it could still darken the photo plate. He tried unsuccessfully to destroy uranium’s activity by dissolving and recrystallizing uranium salt in darkness, a procedure known to destroy phosphorescence in other materials. These first experiments pave the path to the description of natural radioactivity of uranium, a term that Marie Curie will define few years later (“Biographie - Henri Becquerel,” n.d.; Malley, 2011).

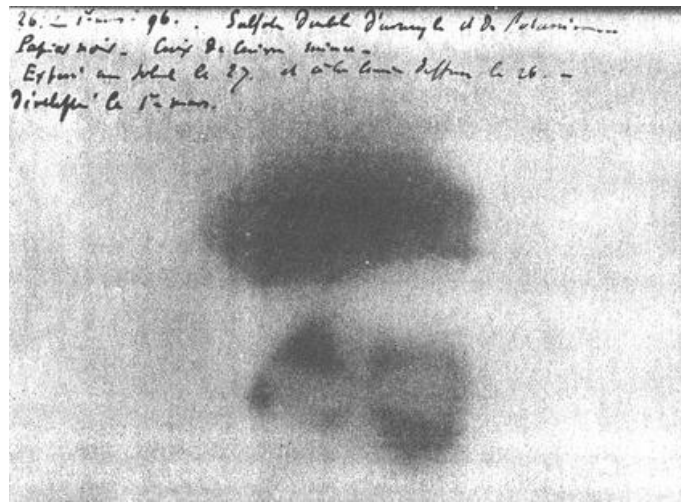


Figure 4 - Photo of uranium salts that stayed in the drawer taken by Becquerel the 1st of march of 1896. From de Andrade Martins, n.d.

- **Marie Curie, Pierre Curie and Radium**

Marie Curie became very interested in Becquerel works and decided to focus her PhD on the uranic rays. First, she discovered that the power of ray emission (that she called activity) was proportional to the amount of uranium contained in the sample. The ray was not influenced by either temperature, chemical or physical state. Herein, the activity was dependent on the uranium element itself. She then tested other elements and found that only thorium and uranium were able to emit invisible rays. She also discovered that two minerals, pitchblende and chalcocite had even higher activity than uranium or thorium thereby demonstrating that these minerals contained unknown elements. This hypothesis was confirmed when she extracted from pitchblende, the polonium element in July 1898, named after her home country and the radium element in December 1898. The latter is about one million more radioactive than uranium and many scientists became interested in this property particularly in the medical field. They experimented on animals that radium could heal wounds and cure malignant tumours. Medical applications ensued from this discovery: therapist could now treat skin cancers by insertion of needles containing radium and even deep tumours using radium pumps.

In 1904, Antoine Bécclère, a French doctor described the harmful effects of radiations after observing redness, burns and ulcers on the medical staff working with radiations to treat cancers. This marks the beginning of radioprotection focusing first on external exposure. Simultaneously, other industrial applications began to grow as radium was advertised in the cosmetic industry as a tonifying and rejuvenating agent added in cream, toothpaste or shampoo. It was also used to prevent and treat arthrosis and rheumatism

and was available off the shelf. Other industrial applications of radium included paints for watch and alarm clock needles phosphorescent in darkness. Female workers used to refine their paintbrush with their mouth after dipping them in radium paint. But in 1924, a new-yorker dentist evoked the emergence of maxillary cancers in these women and correlated it with their profession. This case introduced the notion of radioprotection after internal exposure (contamination) by ingestion (Bayounes et al., 2013; IRSN, 2013; Malley, 2011; Prigent, 2012).

- **Committees on Radioprotection**

In 1928, the International X Ray and Radium Protection Committee is founded and set the first limit of workers exposition to radiation. Later, it will become the International Commission on Radiological Protection (ICRP) that will add some limit exposure to the public population too. In 1955, American atmospheric nuclear assays in the Pacific Ocean, and particularly the consequences on the radioactive fallout on the Japanese Daigo Fukuryū Maru fishermen in 1954, led to the creation of the United Nations Scientific Committee on the Effect of Atomic Radiation (UNSCEAR). Few years later, the International Atomic Energy Agency (IAEA) is founded (1957). These three organizations interact together and aimed at providing recommendations and safety requirements based on scientific knowledge. To do so, the UNSCEAR publishes public reports related on the Effects of Atomic Radiation. These reports serve as review of the scientific knowledge for the ICRP that will further deliver recommendations used by the IAEA that provide safety standards as guides or requirements that can be used as legal basis for governments and decision makers. In France, the ASN (Authority of Nuclear Safety) ensures nuclear safety and radioprotection. The IRSN (Institute for Radioprotection and Nuclear Safety) is the public expert involved

- in UNSCEAR reports with its research activity,
- in ICRP for prospective and retrospective review of the radioprotection standards along with the IAEA (Clarke and Valentin, 2009; IRSN, 2013)
- and the generation of novel knowledge on the effects of ionizing radiations through research programs.

1.1.2. Ionizing radiation: Definitions and biological effects

- α , β and γ rays

In parallel of Curie's work, scientists aimed to identify the different rays emitted by uranium. In 1899, Ernest Rutherford named them alpha and beta radiation and in 1900 Paul Villard distinguished the gamma rays (Malley, 2011).

"Radioactivity is the phenomenon of the spontaneous disintegration of unstable to more energetically stable atomic nuclei" (Guinn, 2003). The release of energy is due to the radioactive decay defined as the decreased number of unstable nuclei. Depending on the nature of the radionuclide, the energy is released under the form of particles (e.g., α , β) or electromagnetic rays (e.g., X, γ). By traveling through matter, the emitted photons and particles can expulse electrons from surrounding atoms: this phenomenon is called ionization. The quantity and track of deposited energy is measured through the linear energy transfer (LET) which is the amount of energy that an ionizing particle transfers to the material traversed per unit distance (eV. μm^{-1}). α particles have a high LET, meaning they transfer most of their energy in short distances, with low penetration in mater (they can be stopped by a sheet of paper). In contrast, β and γ rays have lower LET meaning that they penetrate deeply in tissue but deposit less energy along the ionisation track. β radiations can be stopped by aluminium foil and γ rays by lead plate (**Figure 5**). To quantify the exposure of an organism/tissue to ionizing radiation, the basic quantity is the energy absorbed per unit of mass (J/kg) defined as Gray (Gy) (Delacroix et al., 2006; ICRP, 2008; Park and Kang, 2011; Zimmermann, 2006).

- **Exposure pathways**

Two ways of exposure can occur in the environment: external exposure (the source is located outside the body) or internal contamination (by inhalation, ingestion and contact with the skin). Considering high LET emitter (for instance α particles), the internal contamination is the pathway with more harmful effects.

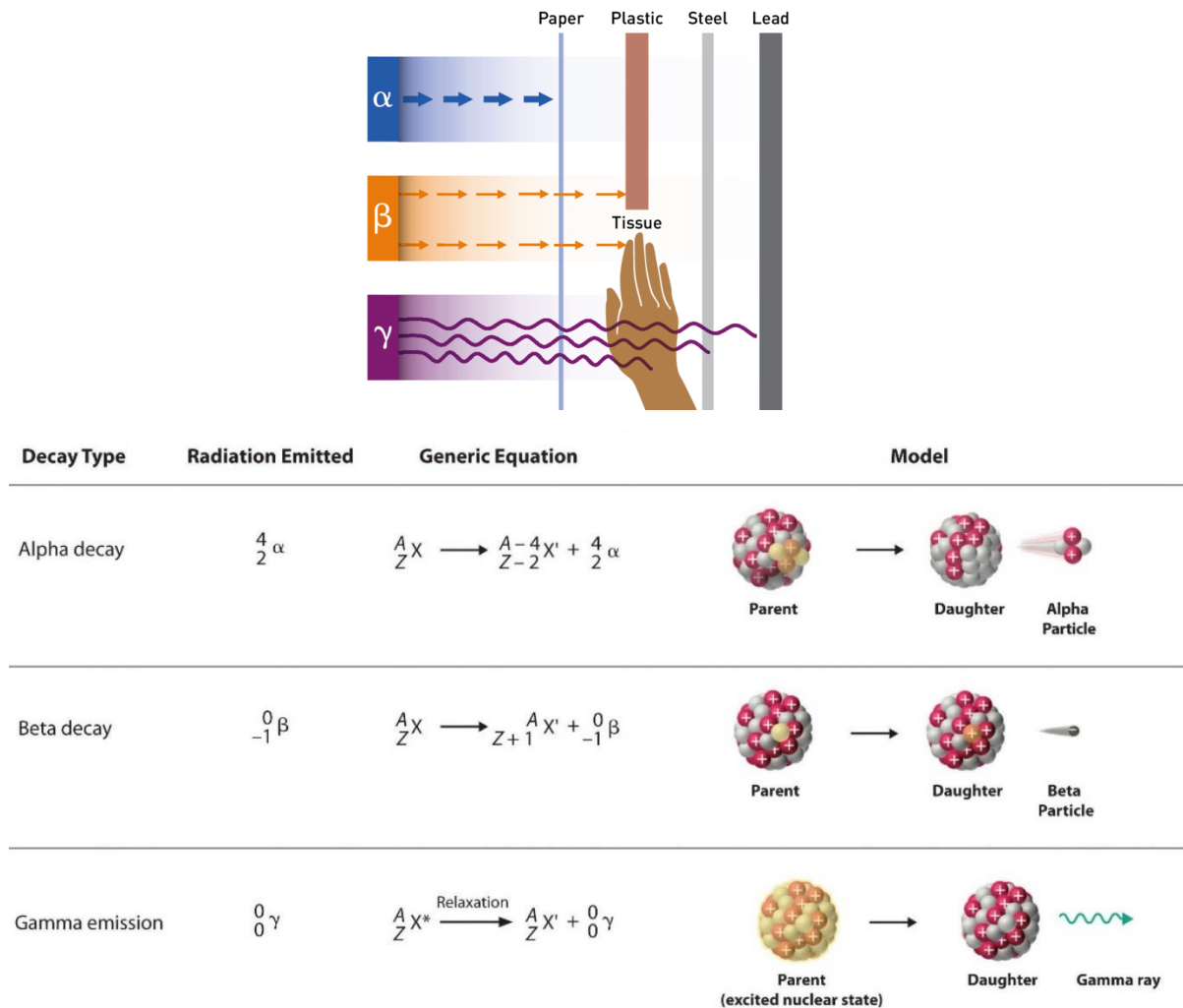


Figure 5 – The penetration power of ionizing radiation (top figure) and modes of nuclear decay (bottom figure). Alpha particles can be stopped by a thick sheet of paper, β particles can be stopped by a one-quarter inch thick sheet of aluminium while γ rays require several inches of dense material (like lead) to shield them. α decay is a type of radioactive decay in which an atomic nucleus emits an alpha particle (helium nucleus) and thereby transforms into a different atomic nucleus, with a mass number reduced by four and atomic number reduced by two. β decay is a type of radioactive decay in which a beta particle is emitted from an atomic nucleus, transforming the original nuclide to an isobar of that nuclide. γ decay is a type of radioactivity in which some unstable atomic nuclei dissipate excess energy by a spontaneous electromagnetic process. From Evans et al., 2016; “14.2,”2022.

- **Advances in Radiobiology allowing the understanding of ionizing radiation mechanisms**

In 1953, Watson and Crick published an article where they proposed the double helix structure of DNA and established the molecular understanding of heredity. This finding had also deep implications in the radioprotection field, as DNA is a major target of ionizing radiation.

Ionizing radiation can produce direct and indirect effects. Direct effects are characterized by the ionization of molecule inducing loss of electrons while indirect effects are caused by the production of free radicals and Reactive Oxygen Species (ROS) in cells via water radiolysis. Under homeostasis, the antioxidant/oxidant status is balanced. However, in the case of high production of free radicals, it is no longer at equilibrium and oxidative stress can harm various targets (lipids, polysaccharides, proteins and DNA).

- Lipids can be targeted by the hydroxyl radical which is known as lipid peroxidation. This phenomenon can disturb membrane permeability and thus cell homeostasis increasing the initial damages caused by radicals (Ayala et al., 2014). Antioxidant systems can act to protect against lipid peroxidation with antioxidant enzymes such as catalase, superoxide dismutase or glutathione peroxidase or non-enzymatic antioxidants (vitamins, selenium, zinc...) (Su et al., 2019).
- Proteins are sensitive to free-radicals aggression which will modify the composition of amino-acids (substitution, depletion, ...) and thus, their secondary and tertiary structures. Oxidized proteins (including carbonylation of side chains) are inactivated causing loss of their biological functions and more vulnerable to proteasome activity.
- Several DNA damages can occur and disturb the integrity of cells: oxidation of nucleotidic bases, abasic sites, simple or double strand breaks, adducts from lipid peroxidation or cross linkage with protein and DNA. Different DNA repair systems can be triggered to respond to these damages (repair pathways through base excision or nucleotide excision, homologous recombination and non-homologous end joining).
- Finally, a less studied process is the oxidation of polysaccharides and for example, glysoxydation was shown to weaken vascular walls. All these mechanisms can lead to various cell damage responses, such as cycle arrest, mutations, mitochondrial dysfunctions and apoptosis. In the case of low LET radiations (i.e. γ rays of Cs-137), the indirect effect through radiolysis is the major process (Averbeck et al., 2020; Desouky et al., 2015; Favier, 2003; Kuzmic, 2018; Reisz et al., 2014).

1.2. The central nervous system of vertebrates and model of choice: *Danio rerio*

Since specific signaling pathways of neurogenesis or targeted cells will be brought up through several of my studies, I will describe the major elements of constitutive and regenerative neurogenesis in the following parts. First, we will focus on general aspects of my model of choice, the *Danio rerio* or usually called zebrafish. Then, the constitutive and regenerative neurogenesis will be detailed. **Constitutive neurogenesis** is defined as the generation of new neurons in normal physiological conditions. In contrast, **regenerative/reactive neurogenesis** is the process by which neuronal production can be re-established or enhanced in the nervous system to restore specific functions after injury (Alunni and Bally-Cuif, 2016).

1.2.1. Characteristics of *Danio rerio*

Zebrafish was first described in 1822 by Francis Hamilton. He belongs to the Cyprinidae family and originates from Asia, lives in freshwater in India, Bangladesh or Nepal (**Table 1; Figure 6**). He resides in stagnant water, low stream rivers and rice fields.

Table 1 – Phylogenetic classification of zebrafish. From Blom et al., 2022.

Kingdom	Animalia
Phylum	Chordata
Class	Actinopterygii
Order	Cypriniformes
Family	Cyprinidae
Genus	Danio
Species	Danio rerio

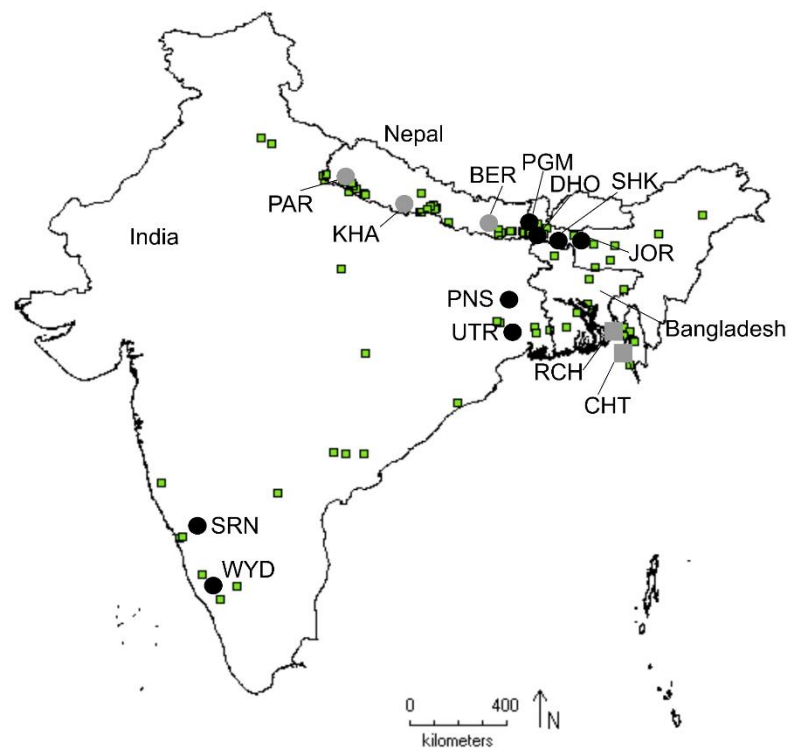


Figure 6 – Sampling locations of wild zebrafish specimen. Black circles correspond to Indian sites, grey circles correspond to Nepali sites, grey squares correspond to Bangladeshi sites, squares indicate the recorded occurrence of zebrafish sourced from the Global Biodiversity Information facility (GBIF) database. From Blom et al., 2022.

His longevity in natural environment is about one year while in laboratory conditions it can reach three years. This vertebrate has many advantages, one being its facility to accommodate to laboratory conditions. Indeed, he can tolerate pHs comprised between 5.9 to 8.1 and temperatures between 18 to 38°C. The optimal temperature is situated between 24 and 29°C. Zebrafish is gregarious, meaning he lives among shoals with several individuals (usually around ten), omnivorous with a diet mainly composed of zooplankton and insects and his length at adulthood is about 4 cm. The huge interest about zebrafish comes from the molecular toolbox developed for this model, including transgenesis, loss and gain of function, and the fact that it is easily amenable to high-throughput screening with 70% of the genes with orthologous sequence in the human genome (Howe et al., 2013). This model is used both in ecotoxicology and in toxicology. However, it is essential to keep in mind that consecutively to an additional duplication of its genome compared to other vertebrate, many zebrafish genes are duplicated compared to mammalian genes which can complicate functional analysis due to gene function redundancy. Zebrafish has been widely used in the field of neuroscience for its ease of use, the conservation of gene

function with mammals and the possibility to carry out behavioral experiments (Blom et al., 2022; Kalueff et al., 2014; Spence et al., 2008).

1.2.2. Constitutive neurogenesis

1.2.2.1. The onset of the central nervous system in vertebrates

Neurulation is the initial step to form the central nervous system in all vertebrates, where the neural tube is created from the neuroectoderm. This pseudostratified epithelium composed of a single layer of neuroepithelial cells derives from the transformation of one part of the dorsal ectoderm that receives inductive signals from the organizer (Boron, 2017; Dafny, n.d.; Purves et al., 2001). The first step in mammals is the invagination of the neural plate forming the neural keel, after closing, the neural rod is formed to finally become the neural tube (**Figure 7A**). In actinopterygians, the telencephalon develops by eversion meaning that the neural tube, in its dorsal region, grows and curves laterally and thus, there is only one medial ventricle comparing to the two lateral ventricles in mammals (**Figure 7B**) (Diotel et al., 2020). The neuroectoderm looks “pseudostratified” because of the process of interkinetic nuclear migration (INM) defined by the nuclear migration towards and away from the ventricular zone during the cell cycle (Götz and Huttner, 2005). The process of neurulation of the anterior neural tube has been described in various species. While few differences can be observed in each animal, the basic steps are conserved. In teleost, the major difference originates from the trunk neural tube that initially forms a solid rod that later develops a lumen.

The anteroposterior axis of the neural tube is patterned via morphogenic gradient, mainly retinoic acid, which will give rise to the anterior brain and more posteriorly, the spinal cord.

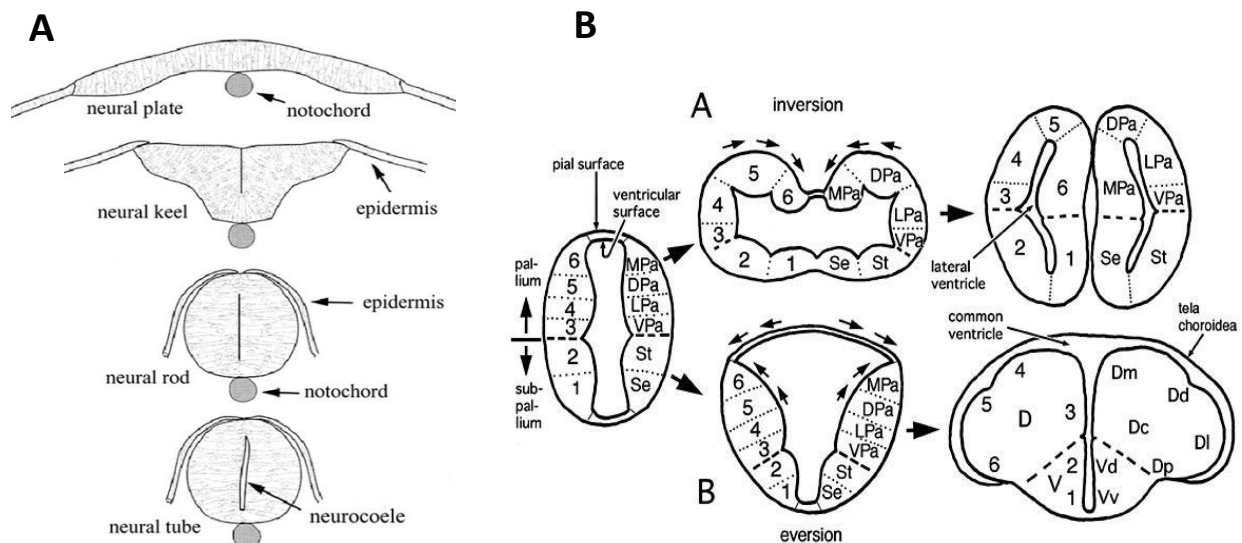


Figure 7 – (A) The neural tube formation, (B) Development of the telencephalon by inversion (mammals) or eversion (teleost). From Geldmacher-Voss et al., 2003 and Yamamoto et al., 2007

The prosomeric model developed in 1993 by Puelles and Rubenstein, propose the segmentation of the neural tube along its anteroposterior axis based on specific molecular cues (Ferran et al., 2022). It describes the generation of distinct brain regions of the neural tube from anterior to posterior: the prosencephalon (forebrain), the mesencephalon (midbrain), the rhombencephalon (hindbrain) and the spinal cord. As embryonic development proceeds, their vesicles further subdivide : the prosencephalon will give rise to the telencephalon (future cerebral cortex and basal ganglia) and diencephalon (future thalamus, subthalamus, hypothalamus and neurohypophysis); the mesencephalon into the tectum and tegmentum; and finally, the rhombencephalon will give rise to the metencephalon (pons and cerebellum) and the myelencephalon (medulla). Regarding the subdivision of the forebrain, a new model as been proposed in which the secondary prosencephalon is divided into three parts, the telencephalon, hypothalamus and optic recess region (ORR) (Editors of Encyclopaedia Britannica, n.d., Yamamoto et al., 2017) (**Figure 8**). In my studies, I focused mostly on the telencephalon, which is thought to be the place where higher cognitive functions such as behavior take place. It is important to note that, several molecular mechanisms for specification of regionalization of telencephalon are conserved in vertebrates. For instance, dorsal and ventral regions of

the telencephalon rely on the fine regulation of *pax6* and *nkx* genes by *shh* in both zebrafish and mice (Chi et al., 2017).

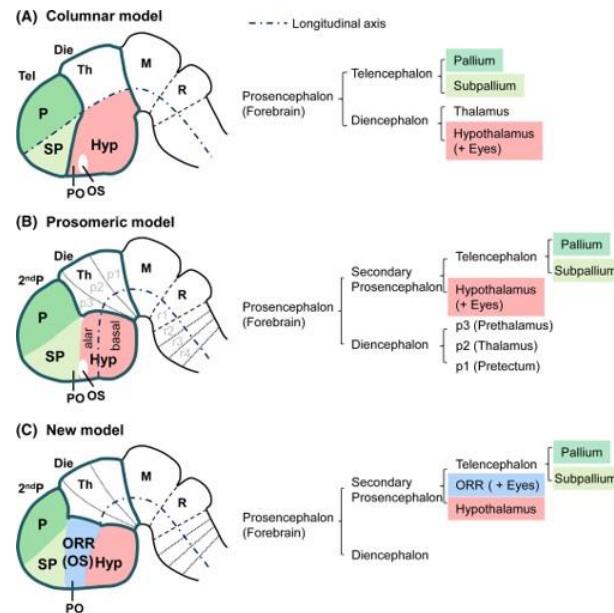


Figure 8 – Different models for the subdivision of the forebrain. (A) The columnar model in which the hypothalamus is considered to be the ventral half of the diencephalon, (B) the prosomeric model proposed by Puelles and Rubenstein, (C) a new model proposed by Affaticati et al.. From Yamamoto et al., 2017

1.2.2.2. Neural/neuronal precursor cells in the mammalian brain

Ernst Haeckel was the first to use the term stem cells in 1868 and referred to them with two meanings: as “the unicellular evolutionary origin of all multicellular organisms” and the “fertilized egg giving rise to all other cell types of the body”. Nowadays, stem cells identify cells that can self-renew and differentiate into different cell types in tissues or organs (Zhao and Moore, 2018). Neural stem cells can self-renew and proliferate to expand their pool without limit and can produce a vast number of different cell types. In contrast, neural progenitor cells have limited self-renewal abilities and differentiate to a more limited repertoire of cell type (neuronal and non-neuronal like astrocytes and oligodendrocytes). Finally, neuronal progenitor cells are committed to the production of neurons.

In mammals, three types of cells can be described as neural stem cells/neural progenitor cells along cortical development or adulthood: the neuroepithelial cells, the

radial glial cells and the type B astrocyte cells. In mammals, these cells differ in terms of localization, time during development, expression of markers, type of divisions and they derived from one another. As previously specified, the initial cells that proliferate by symmetric division are **the neuroepithelial cells** (NECs). Thus, they can expand neural stem cell pools but can eventually give rise to neurons. These cells are in contact with ventricular (apical pole) and pial (basal pole) surfaces with their cell bodies located in the ventricular zone, area next to the ventricles, where neurogenesis occurs during embryonic and fetal development (Kriegstein and Alvarez-Buylla, 2009). At their apical pole they connect to each other by adherens and tight junctions (zonula-occludens-1) (**Figure 9**). Their nucleus follows the interkinetic nuclear migration during the cell cycle: in M phase the nucleus is located at the most apical region and then migrate during G1 phase to undertake the S phase at the basal pole and finally rapidly remove to apical pole during G2 phase. The function of this process was recently understood. Indeed, INM can regulate the exposure to various signals to promote neurogenesis or proliferation such as Notch signaling that can maintain cells in a proliferative state when activated (Del Bene et al., 2008; Götz and Huttner, 2005; Kriegstein and Alvarez-Buylla, 2009; Lindsey et al., 2018).

At the time when neurogenesis begins (around E9-10 in mouse), neuroepithelial cells progressively become **radial glial cells** (RGCs) to replace the latter and thus present morphological changes. These new cells will contribute to neurogenesis until birth. Their cell bodies are still located in the ventricular zone, they remain in contact with ventricular and pial surfaces and also undergo interkinetic nuclear migration at the exception that the movement of the nucleus is restrained to in between the apical surface and the basal limit of the ventricular zone (Götz and Huttner, 2005). Following the progressive thickening of the cortex through corticogenesis, the radial processes of these cells elongate and get microtubules and intermediate filaments such as nestin (also present in neuroepithelial cells), vimentin or in certain species only, the glial fibrillary acidic protein (GFAP).

Progressively, they acquire astroglial markers like astrocyte-specific glutamate transporter (GLAST), brain lipid-binding protein (BLBP), tenascin C (TN-C), S-100 subunit β calcium-binding protein and the glutamine synthase enzyme (GS) (Kriegstein and Alvarez-Buylla, 2009). In contrast to neuroepithelial cells, radial glial cells only anchored to each other through adherens junctions, are in contact with blood vessels and divide

asymmetrically to produce one radial glial cell and one neuron directly or via an intermediate progenitor cell (**Figure 9**).

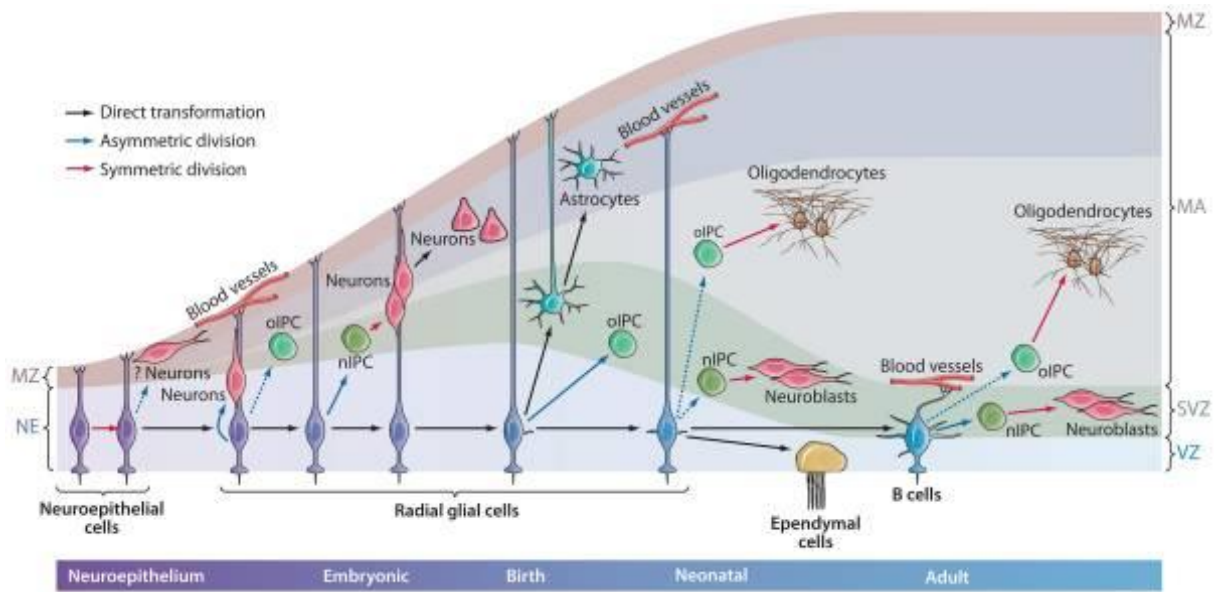


Figure 9 - Neural stem cells in development and in the adult. MA: mantle, MZ: marginal zone, NE: neuroepithelium, nIPC : neurogenic progenitor cell, oIPC : oligodendrocytic progenitor cell, RG : radial glia, SVZ: subventricular zone, VZ: ventricular zone. Solid arrows are supported by experimental evidence, dashed arrows are hypothetical. From Kriegstein and Alvarez Buylla, 2009.

After birth, radial glial cells can produce neurons, ependymal cells and **type B astrocyte** cells that will, for some of them, ensure a progenitor role in adult neurogenesis in the subventricular zone of the lateral ventricles, one of the main neurogenic niches in the mice adult brain. The latter cells can thus indirectly through intermediate progenitors give rise to oligodendrocytes but also to neuroblasts that will become neurons after migration. Another neurogenic niche, extensively studied these past years, is the subgranular zone of the dentate gyrus of the hippocampus composed of radial glial cells also called type 1 cells. These two zones are called neurogenic niches because stem cells reside in these niches and depend on them by the microenvironment they provide: regulation of NSC survival and balance between quiescence and production of progeny (Ceci et al., 2019).

1.2.2.3. Constitutive neurogenesis in adult zebrafish

At the beginning of the last century, Ramon y Cajal studied in detail the human brain anatomy and reported that neurogenesis was restricted to the developmental period (De Castro, 2019). However, in 1962, Altman discovered newly born cells in the adult rodent brain and few years later, this assumption was confirmed and demonstrated by the

presence of new-born neurons in the adult brain of mice and canaries (Goldman and Windrem, 2006; Kaplan and Hinds, 1977). Afterwards, with the development of new technologies (BrdU or viral vectors labelling), the field of adult neurogenesis took off. The presence of constitutive neurogenic niches has thus been demonstrated in adult rodents (rabbit and mouse) (Alvarez-Buylla and Lim, 2004) or in humans (Williams, 2014) but mammals' brain have limited adult neurogenesis capacity compared to birds, reptiles (Ceci et al., 2019) or teleosts (Ekström et al., 2001; Grandel et al., 2006; Grandel and Brand, 2013; Zupanc, 2001). In zebrafish brain, neurogenic niches (around sixteen) are mainly disposed along the ventricular layers of the telencephalon, diencephalon and rhombencephalon (**Figure 10**).

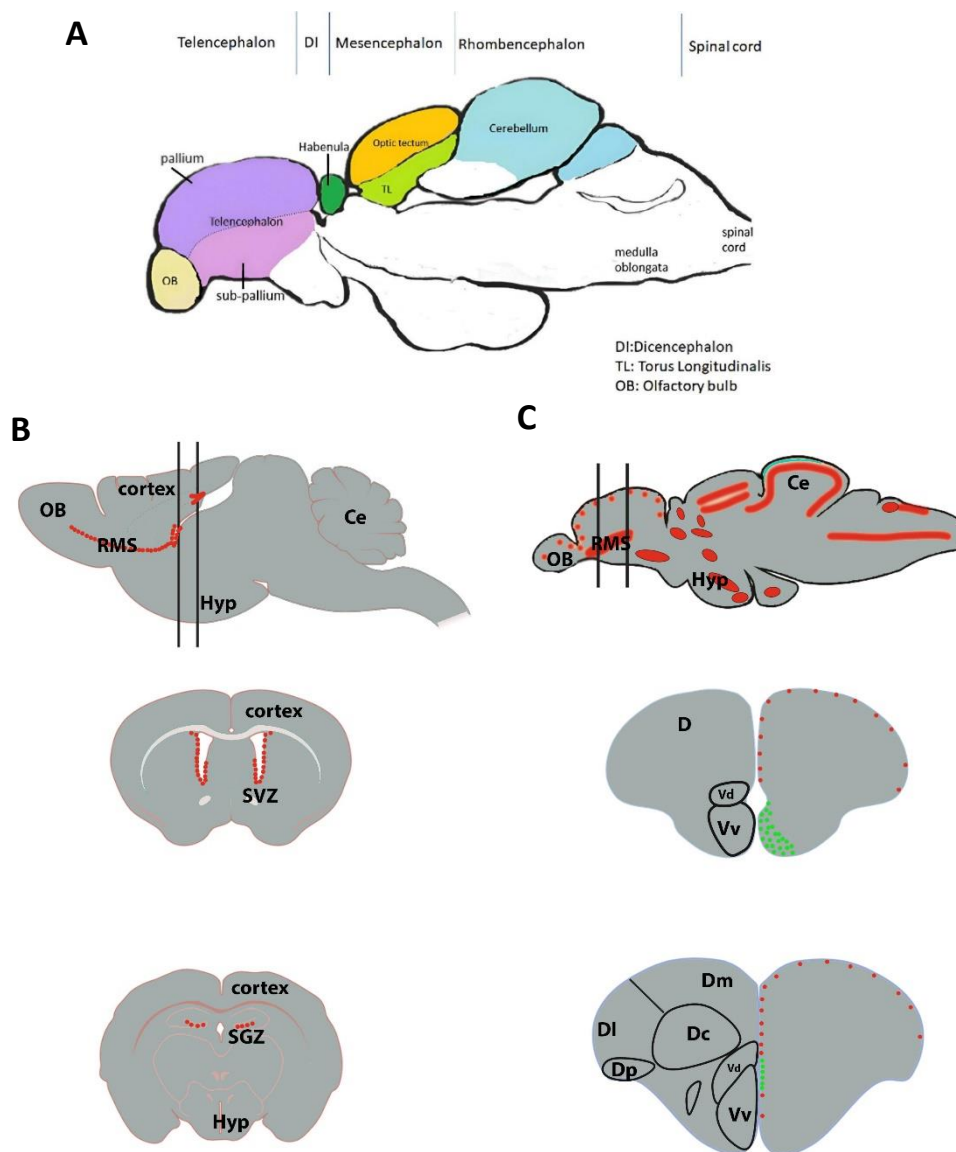


Figure 10 - Sagittal section of zebrafish brain (A) and neurogenic niches in the brain of mammals (B) and zebrafish (C). Red dots represent the proliferative area in mammals and slow cycling progenitors (type II cells) in zebrafish. Green dots represent fast cycling cells. From Shenoy et al., 2022; Diotel et al., 2020.

Here we will focus on two neurogenic niches located in the telencephalon: the dorsal telencephalon (pallium) and the ventral telencephalon (subpallium). The telencephalon is of particular interest since many homologies were suggested with the mammalian brain in terms of expression of transcription factors or enzyme synthesizing neurotransmitters. As an example, specific markers for GABAergic neurons or serotonergic innervation of the telencephalon are similarly expressed in rodents' telencephalic regions (Diotel et al., 2020; Kumamoto and Hanashima, 2014). If some regional similarity exists during development, one can observe a surprising variability of shape, size and connectivity in the adult brain. Thus, if some homology can be described between zebrafish and mouse brain organisation, it is not possible to define one to one functional equivalence. These homologies thus need to be confirmed since many controversies were depicted and thus interpretation must be carefully described.

- **Neural precursor cells in the telencephalon**

Similarly to brain mammal's development, zebrafish neural progenitors also reside in the ventricular zone and have the same radial morphology. Their cell bodies are in the ventricular zone and radial processes cross throughout the parenchyma. In the telencephalon of adult zebrafish, three types of neuronal precursors can be identified (**Figure 11**): Radial glial cells which include Type I cells, Type II cells and Type III cells. Type I cells correspond to quiescent neural stem cells and thus express radial glial markers ($S100\beta$, Gfap, Blbp, Aro-B, and Vimentin) and the progenitor markers Sox2 and Nestin. Under constitutive neurogenesis (physiological conditions), most cells stay in a quiescent state. When Notch signaling is inhibited, Type I cells convert into activated neural stem cells (Type II cells). They contain the same set of markers as Type I in addition with proliferating cell nuclear antigen (Pcna), a marker of proliferation. RGCs (Type I or Type II) are not homogeneous since they do not all co-express the same markers. For example, it was demonstrated that some RGCs co-express Aromatase B and Blbp however others only express aromatase B or Blbp (Diotel et al., 2016). Moreover, additional markers were observed in radial glial cells (mainly in quiescent cells) such as the inhibitor of DNA binding 1 (*id1*) or the chemokine receptor *cxcr4* well known for their role in regenerative neurogenesis (Ceci et al., 2019; Diotel et al., 2020).

Type I and II cells can go through direct conversion into neurons and symmetrical division (two radial glial cells) respectively to expand the neural stem cell pool but the majority of them divide asymmetrically thus maintaining the pool and generating new neuroblasts (Type III) (Barbosa and Ninkovic, 2016; Zambusi and Ninkovic, 2020). Type III cells represent one-third of Pcn^a cells (Ceci et al., 2019) and also express the polysialylated-neural cell adhesion molecule Psa-ncam, characteristic of early neuronal differentiation. They are subdivided into two subsets: Type IIIa and Type IIIb, the latest being negative for radial glial markers but positive for Sox2, Pcn^a and Psa-ncam. These cells perform symmetric divisions.

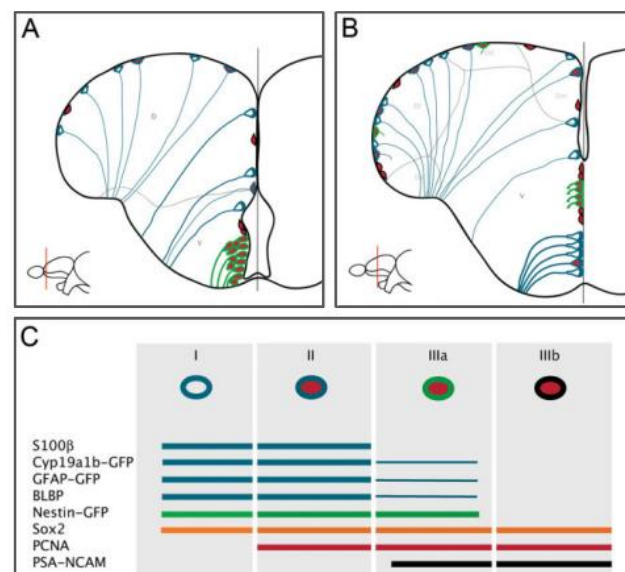


Figure 11 – Different cell types at the ventricular zone of the telencephalon. (A) Anterior and (B) medial coronal sections of the telencephalon. (C) Markers of expression of quiescent radial glial cells (Type I), activated radial glial cells (Type II) and neuroblasts (Type III). From März et al., 2010.

- **The subpallial telencephalon**

The subpallial telencephalon comprises a higher proliferation state in comparison to the pallial domain. Two brain areas subdivide the ventral telencephalon: the ventral nucleus (Vv) which was suggested as the homolog of the subventricular zone of the lateral ventricle of mammals and the dorsal nucleus (Vd) which presents more similarities with the dorsal telencephalon in terms of proliferation zones and expression of markers typical of radial glial cells (Wullmann et al., 1996) (**Figure 11C**).

In the Vv, progenitors present neuroepithelial-like characteristics such as interkinetic nuclear migration but not or barely radial glial markers ; however they express Nestin

(Ganz et al., 2010; März et al., 2010). Adolf and his team demonstrated the presence of fast cycling progenitors grouped in a wide pack (Adolf et al., 2006); they are considered as neuroblasts that migrate toward the olfactory bulb through a rostral migratory stream-like in homology of the rostral migratory stream (RMS) found in rodents at the exception that in fish the cells migrate along blood vessels instead of glial tubes (Diotel et al., 2020; Kishimoto et al., 2011). The newly generated neurons are differentiated into Gabaergic and Th+ (Tyrosin hydroxylase +) interneurons and are located periventricularly (Ceci et al., 2019; Zambusi and Ninkovic, 2020). Other neurogenic niches contain these neuroepithelial-like cells such as the optic tectum and the cerebellum.

- **The pallial telencephalon**

More brain regions constitute the dorsal telencephalon with a central zone (Dc), a dorsomedial zone (Dm), a lateral zone (Dl) which is equivalent to the SGZ of the DG and a posterior zone (Dp) (Diotel, 2020). Except for the posterior zone, the pallium is mainly composed of slow cycling progenitors (Adolf et al., 2006; März et al., 2010) (**Figure 11**) that present radial glial features (Gfap, S100b, vimentin, Glutamine synthetase (GS), aromatase B (Aro-B)) (Ganz and Brand, 2016) and are grouped by small clusters along the ventricular surface. Contrastingly to the subpallial domain, new neurons don't migrate far away but stay closely to the ventricle. Recently, neuroepithelial progenitors were discovered in the lateral edge of the pallium that contributes to the generation of RG and neurons in the Dl. These cells are apico-basally polarized, are proliferating and don't express astroglial markers which distinguish them from RG (Labusch et al., 2020; Than-Trong et al., 2018).

- **Regulation of constitutive neurogenesis**

Many signaling pathways that control cell quiescence, proliferation and differentiation are involved in both adult mouse and fish neurogenesis. In zebrafish, **Fgf signaling** is involved in proliferation of progenitors. Indeed, it was shown that blockade of this pathway decreased the number of proliferating cells in the Vv where *fgf3* and *fgf8* are expressed (Ganz and Brand, 2016). As previously mentioned, **Notch signaling**, in contrast to mammals, maintains quiescence state of Type I cells. This was proposed by blocking Notch signaling in the dorsal telencephalon by Chapouton and his team. They observed an increase in proliferation partially mediated by *notch3* and contrastingly, when Notch signaling is ectopically activated, the progenitors quit the cell cycle (Chapouton et al.,

2010). However, maintaining a quiescent state is not restricted to Notch signaling (Ganz and Brand, 2016) since not all cells proliferate when Notch is activated. More precisely, *notch3* was described to play a dual role by promoting both quiescence and the proliferation of RGCs via two downstream mediators, members of the **Hairy enhancer** of split transcription factor family, *her4* for quiescence or *hey1* for proliferation. *notch1b* on the other way is necessary for self-renewal (Alunni et al., 2013). In the absence of HES/Her proteins, proneural genes can induce the neurogenic fate such as through **achaete-scute like** (*Ascl*). In adult mouse neurogenesis, it was shown that these proneural genes can be constantly expressed or oscillating under the control of *Hes/her* genes expression which oscillates itself. This mechanism integrating the oscillations of *Hes/her* with *Ascl1* expression could explain the dual role of Notch signaling (Sueda et al., 2019). Indeed, in quiescent NSCs, cells are maintained at this stage because *Hes1* is kept constantly at a high level and thus the expression of *Ascl1* is repressed. In activated NSCs, since *Hes* (ortholog of *her*) autoregulatory repression is not inhibited it leads to the oscillation of *Ascl1* and *Hes1*. Finally, when Notch and thus Hes signaling is repressed, *Ascl1* is constantly expressed at high levels bringing to the differentiation into neuroblasts (**Figure 12**). We suppose that the same mechanisms could occur in zebrafish since all of these genes are expressed in the ventricular zone of the telencephalon (Diotel et al., 2020; Ganz and Brand, 2016). **Inhibitor of differentiation** (*Id*) are target genes of the Bone morphogenetic proteins (BMPs) which are members of the transforming growth factor β (TGF- β) family and known to regulate developmental and adult homeostatic processes (Bier and De Robertis, 2015; Wang et al., 2014). It was demonstrated that *Id1* controls stem cell proliferation by keeping NSCs in quiescence through the interaction with *Hes1* during mouse neurogenesis leading to the repressed expression of *Ascl1* (Bai et al., 2007). In adult zebrafish, *Id1* preferentially interacts with Her4, 6 and 9 proteins during constitutive and regenerative neurogenesis (**Figure 12**).

Transcriptomics in zebrafish can bring knowledge as for characterizing specific cell types through their exact expression genes profile and thus to improve discrimination between cell types as well as understanding the mechanisms under the specification of cell fate. For example, it was demonstrated that pathways involved in transcription, translation, DNA replication and DNA repair and cell cycle progression are downregulated while cell-cell communication, cell adhesion (Codega et al., 2014; Ju Shin et al., 2015), cell signaling and lipid metabolism (Llorens-Bobadilla et al., 2015; Than-Trong et al., 2018) are upregulated in NSC quiescence.

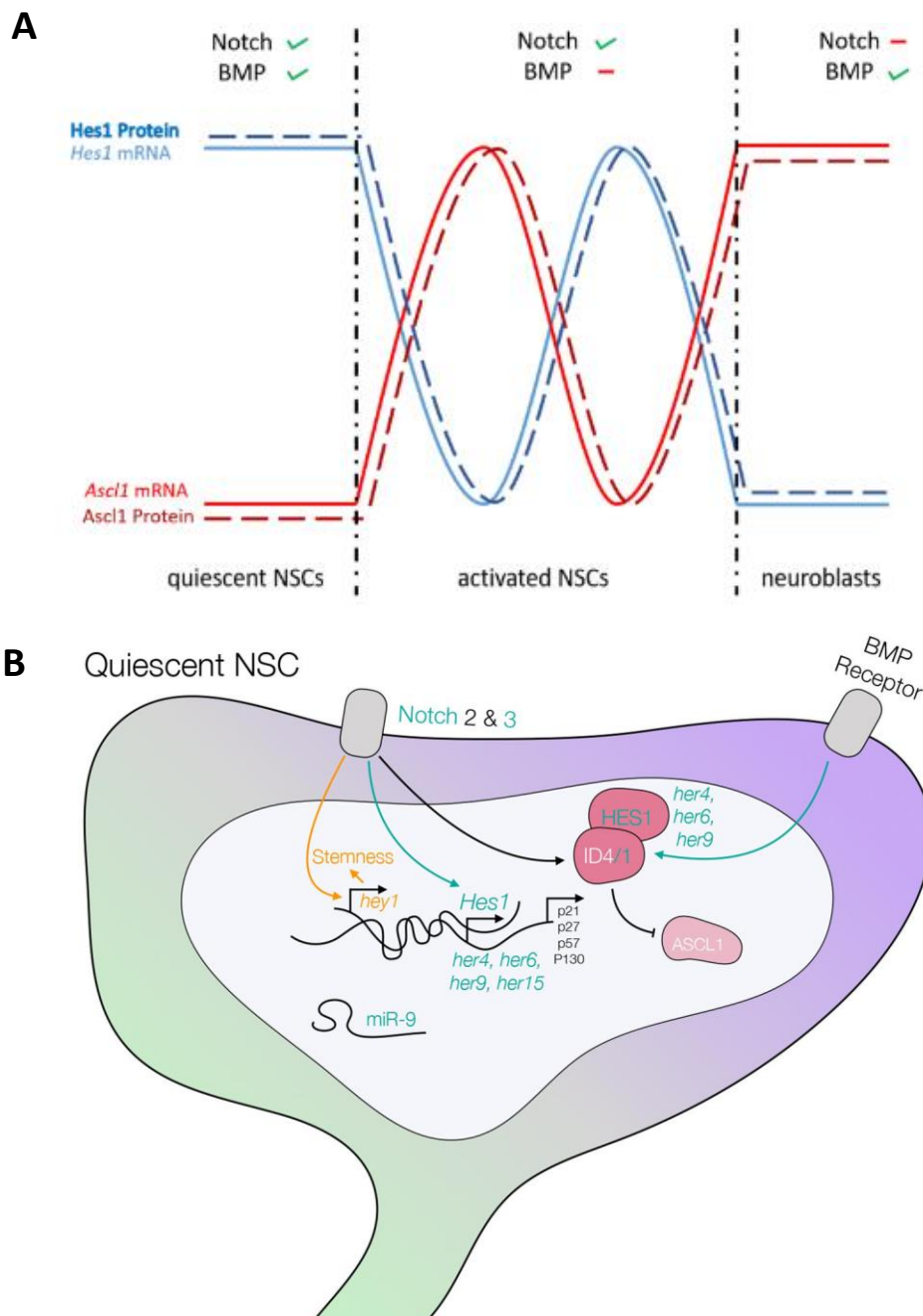


Figure 12 - (A) Expression of key genes for the regulation of cell states quiescent NSCs, activated NSCs or neuroblasts in mouse. When Bmp and Notch signaling are active, the negative autoregulation of *Hes1* is high, inducing *Hes1* and *Ascl1* expression which oscillates and leads to activation and proliferation of NSCs. When Notch signaling is not active, the repression of *Hes1* on *Ascl1* is inactive causing the development of NSCs into neuroblasts. From Diotel et al., 2020 (B) Signaling pathways to maintain quiescence in NSCs. In green and orange are represented the demonstrated pathways in zebrafish. Data confirmed in mouse but still needs demonstration in zebrafish are depicted in black. From Labusch et al., 2020.

1.2.3. Regenerative neurogenesis

1.2.3.1. The overall process

Regeneration is defined as the restoration of organ homeostasis after traumatic injury and can occur at multiple biological levels from cellular, tissue to organ or the whole-body organism. This implies a strict regulation to coordinate the replacement of various cell types from pre-existent cells. The ability of regeneration is not found in all animals as nematodes or leeches cannot regenerate any structure and, within the same organism, regeneration capacity is not equivalent in all body part as for instance annelid worms can regenerate their tail but not their head. In an ecological context, regeneration can be assimilated as a protective process against predators, for example the alligator lizards (*Elgaria coerulea principis*) autotomize (self-amputation) his tail to escape from predators (Bely and Nyberg, 2010). Such capacity (lost in mammals but present in amphibian), and teleost fish, was associated with the constant growth of these organisms during their life, including adulthood. Zebrafish has become an excellent model to study the processes of regeneration. Indeed, various organs can self-regenerate as fins, heart, lateral line, pancreas or central nervous system. The well-functioning of the central nervous system (CNS) is crucial to maintain vital mechanisms as the control of movement, cognitive functions and other behaviors as well as the regulation of the endocrine system. Zebrafish can regenerate neurons within the retina, the spinal cord, and the brain (telencephalon, optic tectum, cerebellum) from pre-existent radial glial cells (Gemberling et al., 2013; Kizil et al., 2012b).

Regenerative neurogenesis requires:

- presence of progenitors (constitutive or latent) to differentiate in cell types that were loss because of the injury.
- A sufficient proliferation of these progenitors to maintain a pool of progenitors.
- Capacity of these progenitors to migrate towards lesion site.
- Regulation of the cell specification and differentiation processes to restore the function of the loss structure.

Several methods can be used to induce regenerative neurogenesis: chemical compounds, transgenic approaches for cell ablation or physical lesion which is the most common used technique (Kizil et al., 2012b). Stabwound injury is a mechanical method that can be performed by the insertion of a needle through the skull into the telencephalon

or through nostrils. In the first case, the dorsal part of the ventricular zone and the parenchyma are damaged causing loss of radial glial stem cells and neurons. In the second case, the damage is restricted to the parenchyma. Interestingly, newly generated neurons are localized deeply into the parenchyma which is not observed with constitutive neurogenesis. In contrast to mammals where a glial scar persists, the new neurons produced during the regeneration integrate functionally the existing neuronal network (Zambusi and Ninkovic, 2020). Regenerative neurogenesis is characterized by a cascade of events that start with death of damaged cells, a process that is solved in 1 to 3 days post lesion (dpl) (Ghaddar et al., 2021). Then, activation of the immune system induces acute inflammatory responses and the removal of dying neurons (Kyritsis et al., 2012). Afterwards, the proliferation of radial glial cells with a peak at day 7 (Ghaddar et al., 2021; Kishimoto et al., 2012) allows the production of neuroblasts that will migrate along the radial glial processes towards lesion site and differentiate into mature neurons.

Interestingly, live imaging of adult neural stem cell showed that after injury, asymmetric division of radial glial cells and symmetric division generating two neuronal precursors are frequently observed, thus depleting neural stem cell pool over time. This is specific to regenerative neurogenesis since it is not observed in the intact brain (Barbosa et al., 2015). These processes are complemented by direct conversion of stem cells into neurons, a process under the control of the *aryl hydrocarbon receptor (ahr)* that synchronizes direct conversion with inflammatory state of the brain (Di Giaimo et al., 2018; Zambusi and Ninkovic, 2020). Indeed, low AhR signaling shortly after wounding (2 days post lesion) was seen to support stem cell proliferation while high levels achieved at 7 days post lesion promotes direct conversion.

1.2.3.2. Regulation of regenerative neurogenesis

The activation of specific molecular pathways involved in inflammation and constitutive neurogenesis is mandatory for zebrafish telencephalon regenerative neurogenesis. These signals allow the formation of new neurons, their migration, differentiation and integration within the damaged telencephalic hemisphere (**Figure 13**).

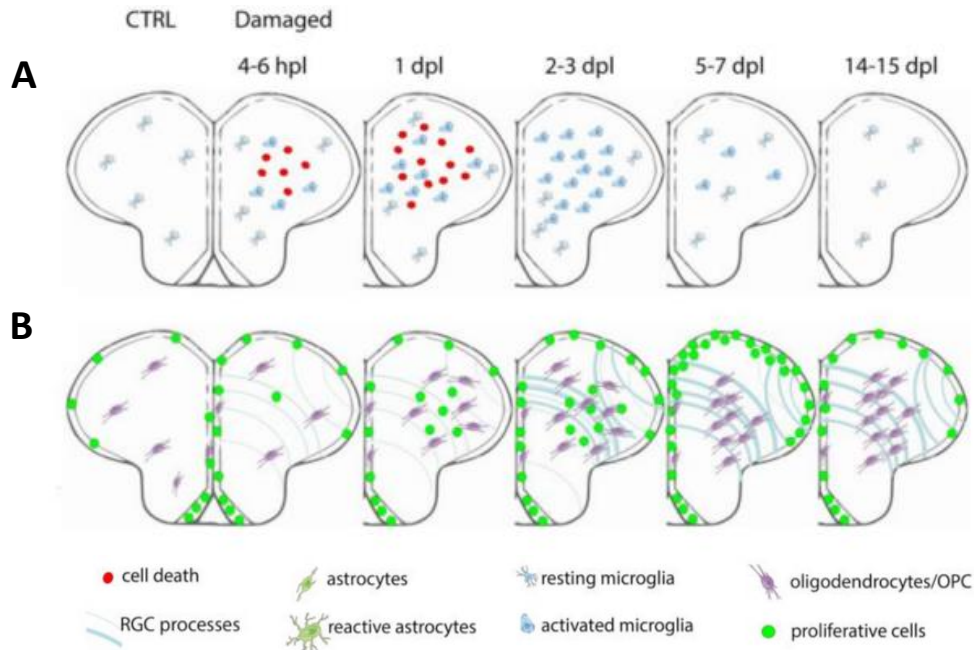


Figure 13: Cellular events occurring after stabwound injury in the telencephalon of zebrafish. (A) death of neurons, microglial recruitment and activation of the immune system. (B) Proliferation of RGCs with hypertrophy of processes and recruitment and response of oligodendrocytes/OPCs. Hpl: hours post-lesion, dpl: days post-lesion. From Ghaddar et al., 2021

- **Inflammatory signals**

The first response to traumatic injury is cell death quickly followed by the activation of inflammatory processes that last from 6hpl to 7dpl approximately (Ghaddar et al., 2021; Zambusi and Ninkovic, 2020). Demirci and his team showed that Wnt/ β -catenin signaling is involved in the up-regulation of apoptosis genes (*apaf1*, *baxa*, *traf4b*, *casp3a*) during early brain regeneration (Demirci et al., 2020). After lesion, microglia is activated to act at the injury site and the mobilization of microglial cells toward the injury site is controlled by long-range Ca^{2+} waves activating ATP signaling-dependent chemotaxis, through the purinergic P2Y₁₂ receptor (Haynes et al., 2006; Sieger et al., 2012). Leukotriene C₄ (Ltc₄) signaling, as part of the inflammatory response, was shown to be sufficient to initiate regenerative response (Cosacak et al., 2015; Kyritsis et al., 2012) and activate radial glial cells. The activated immune cells remove dying neurons, can release pro-inflammatory cytokines (IL-1 β and IL-6), the tumor necrosis factor (TNF- α) as well as anti-inflammatory factors, such as Tgf- β and the cytokines Il-4 and Il-10. Specifically in zebrafish telencephalon regenerative neurogenesis, only the role of Il-1 β , Il-8 and Tnf- α was currently demonstrated (Ghaddar et al., 2021; Kyritsis et al., 2012). For example, using liposomal clodronate and colony stimulating factor-1 receptor (Csf1r) inhibition to

ablate microglia during telencephalon injury caused inhibition of regenerative neurogenesis. Thus, Kanagaraj and colleagues concluded that activated microglia is involved in the induction of Tnf- α expression which stimulates Stat3 and β -Catenin signaling to promote proper amplifying intermediate progenitor cell amplification and neurogenesis (Kanagaraj et al., 2020).

- **Proliferation/maintenance of the pool of neural stem cells and generation of new neurons**

Compared to the mammalian brain where inflammatory response is prolonged, in zebrafish CNS, this initial phase is quickly resolved and followed by proliferation of RGCs. The zinc-finger transcription factor *gata3* was found to be expressed in non-proliferating RGCs within the first day after lesion (12hpl), becomes predominant in the proliferating RGCs at 3 dpl, and persists in the daughter new-born neurons at 7 dpl. This confers roles of *gata3* in promoting RGCs proliferation, in neurogenesis and migration of neuroblasts that were demonstrated by *gata3* knockdown. Interestingly, *gata3* is not expressed in constitutive neurogenesis (Cosacak et al., 2015; Kyritsis et al., 2012) and its expression is induced by the LTC4-CysLT1 pathway meaning that activation of the inflammation process is mandatory for its induction (Kyritsis et al., 2012). In addition, using heterozygous heat-shock-inducible dominant negative Fgfr1-EGFP transgenic fish, Fgf signaling was demonstrated as a requirement for induction of *gata3* expression, however the exact pathways of regulation in the telencephalon were not investigated yet (Kizil et al., 2012c). In spinal cord regeneration in zebrafish, *fgf3*, *fgf8a* and target genes as *spry4*, *pea3* and *erm* were showed to be highly expressed by neural progenitors (Goldshmit et al., 2012). Expression of transcriptional regulator *id1* is also upregulated in neural stem cells after stabwound lesion and is independent of inflammatory signals (Viales et al., 2015) but depend on Bmp signaling pathway (Zhang et al., 2020). It was suggested that its function is to avoid the depletion of the neural stem cell pool by maintaining them in a quiescence state and that this response is delayed in comparison to *gata3* (Diotel et al., 2020; Lindsey et al., 2018). Consequently, Id1 is mainly expressed in type I cells and by a little proportion of type II cells progenitors (Zambusi and Ninkovic, 2020). In the unlesioned brain, Notch is known to be involved in promoting NSCs quiescence. In the same way, the blockade of Notch 3 receptor upregulation after traumatic injury demonstrated an increase in type II progenitors and a decrease in quiescence type I cells, showing that Notch 3 fulfills the same role as in constitutive neurogenesis which is

maintaining the cell quiescence state. Conversely, Notch 1 receptor has an opposite function as in constitutive neurogenesis. After lesion, it acts in promoting RGCs activation and proliferation (Kishimoto et al., 2012; Zambusi and Ninkovic, 2020). However, the exact involvement of BMP pathway and a possible interaction with Notch signaling to promote quiescence using similar mechanisms as in constitutive neurogenesis was not elucidated yet and thus cannot be excluded.

The chemokine receptor *cxcr5* was identified as expressed in RGCs and neurons and suggested with a permissive role for regenerative neurogenesis with an involvement in enhancing RGCs proliferation as well as sufficient and required for production of newborn neurons (Kizil et al., 2012a). In mammals, *Cxcr5* is expressed in mature B cells and in a subset of T cells in the immune system. The chemokine CXCL13 was demonstrated to mediate neuroinflammation through this receptor particularly in amyotrophic lateral sclerosis to preserve motoneurons (Trolese et al., 2020). It is also found in granule and Purkinje cell layer of the cerebellum in adult mice however its role in adult neurogenesis and even more regenerative neurogenesis in vertebrates still needs investigation.

- **Migration and survival**

Prokineticin 2 (*Prok2*) and Sprouty-related EVH1 domain containing 2 (*Spred2*) are involved in migration and survival of neuronal progenitors at the lesion site. Moreover, *Prok2* was suggested to first act as a chemoattractant to drive migration and later as a neurotrophic factor (Ayari et al., 2010). Newborn neurons employ radial glial processes to migrate to their target site (Kishimoto et al., 2012; Lim et al., 2016). The molecular mechanisms underlying cell survival were not investigated yet however it was proposed that the glial scar due to long-term accumulation of glial cells in mammalian brain is first beneficial to limit the injury of the tissue but later detrimental inducing a unfavorable environment for neuron survival (Adams and Gallo, 2018; Baumgart et al., 2012).

- **Oligodendrocytes and axonal regeneration**

Oligodendrocytes are essential glial cells since they myelinate axons in the central nervous system which provides electrical potential insulation for axonal conduction. The recruitment of oligodendrocytes and oligodendrocyte progenitor cells (OPCs) during regeneration was investigated and showed recruitment of oligodendrocytes from 1 dpl to 14 dpl (März et al., 2011). In this study, some proliferating oligodendrocytes positive for *Pcna* and *oligo2:EGFP* were detected at the lesion site but no significant increase in the

proliferation rate of Oligo2:EGFP was detected in comparison to the uninjured hemisphere, in contrast to mammals. Contrastingly, one study demonstrated that Olig2+ RG cells participate in the regeneration of oligodendrocytes in the injured brain along with recruitment of OPCs present in the parenchyma of the injured telencephalon (Kim et al., 2020). Moreover, this study aimed at evaluating the role of Notch signaling in the recruitment of OPC after lesion through its inhibition by treating with LY-411575 which impairs Notch activation. This blocking induced the proliferation of OPCs and RG Oligo2+ in both hemispheres of the telencephalon (uninjured and injured) thus demonstrating the involvement of Notch signaling pathway in OPC proliferation. In addition, Rosario Sanchez-Gonzales and colleagues showed that cerebrospinal fluid-derived molecules, unknown at this time, driving the reactive gliosis are able to activate the toll-like receptor 2 (Tlr2) and the chemokine receptor (Cxcr3) innate immunity pathways which promote the increase of OPCs proliferation independently from microglia and invading monocytes even if their role is not excluded since these receptors are expressed in various cell types (microglia, oligodendroglia, neurons...)(Sanchez-Gonzalez et al., 2022).

The process of axon regeneration was mainly described after spinal cord or optic nerve injury in zebrafish (Becker and Becker, 2014). After spinal cord injury, it has been described that the chondroitin sulfate proteoglycans (CSPGs) drive the regeneration by preventing aberrant connections of nerve axons (Fawcett and Asher, 1999; Becker and Becker, 2002; Vajn et al., 2013). Bridges formed by radial glial cells to re-connect the severed spinal cord were suggested to support axon growth (Goldshmit et al., 2012). In addition, the generation of a permissive environment for axonal regrowth was proposed including the upregulated expression of genes like *atf3*, *fgf2*, *mmp-9*, *stat3*, *socs3* and *sox11* (Hui et al., 2014). Furthermore, molecules known for axonal pathfinding during developing nervous system with repellent or attraction properties, involved in cell adhesion or in organization of axons were found to be upregulated from 3 days post injury (dpi) to 10 dpi (CAMs, semaphorins, netrins, robo, slit, ephrin and rtk) (Devine and Key, 2008; Roos et al., 1999; Winberg et al., 1998; Xu et al., 1996). Finally, calcium transients were proposed to be associated with axonal regeneration and play a role in resealing the ruptured membrane for growth cone formation (important step during initiation of axon regeneration). *atf3* was also proposed to be an important mediator in optic nerve regeneration as it was upregulated in the ganglion cell layer of the retina, the nerve fiber layer and the optic nerve of the injured eye (Saul et al., 2010).

Altogether, these results show that regenerative neurogenesis in CNS zebrafish relies on the re-activation of developmental signals and on added processes (e.g. expression of *gata3*) specific to injury. Moreover, it was demonstrated the existence of interactions between the unlesioned and lesioned hemispheres with many shared dysregulated genes as for Wnt signaling genes at 20 hpl (Demirci et al., 2020)

1.2.4. Ionizing radiation and the central nervous system

Morphological effects were described in wild species following Chernobyl and Fukushima accidents. Indeed, analyzing birds of the contaminated areas in Chernobyl, a negative correlation between relative brain size and level of background radiation was brought to light in yearling birds (Møller et al., 2011). In another study, Hayama and his team measured the relative growth of 31 fetuses of *Macaca fuscata* conceived prior to Fukushima Daiichi accident and 31 fetuses conceived after the disaster and demonstrated that the head size (measure of the occipital frontal diameter and biparietal diameter) was significantly lower in fetuses born after the accident (Hayama et al., 2017). Such developmental effects on brain size (microcephaly) were also described in human exposed *in utero* after the atomic explosion in Hiroshima and Nagasaki atomic bomb (Folley et al., 1952). The frequency and severity of microcephaly was shown to be dose-dependent in epidemiological data and animal experiments however the dose-threshold between humans and mice vary (above 0.3 Gy in the mouse (Verreet et al., 2016) and above 0.1 Gy for the human (Otake and Schull, 1998; Jaylet et al., 2022). Finally, acute exposure of zebrafish 6 hours post fecundation (hpf) embryos to 0.5 Gy induced apoptosis in the forebrain (Bladen et al., 2005) at 24 hpf and exposure to 4 Gy at 4 hpf induced smaller brain at 72 hpf (McAleer et al., 2005).

At a cellular scale, studies aimed to understand the impact on neurogenesis after radiation. A reduction of neurogenesis caused by depletion of progenitors (neural stem cells or neuroblasts) was described in the dentate gyrus and the SVZ of rodents after acute exposure of nine-day-old or adults at 3 Gy, 6 Gy or 10 Gy (Achanta et al., 2012; 2006; Hellström et al., 2009).

The mechanisms contributing to stem cell dysfunction and thus reduction of neurogenesis at acute high doses of IR are apoptosis, inhibition of cell-cycle progression by oxidative stress or DNA double strand breaks (Acharya et al., 2010) and neuro-inflammation (Belka et al., 2001; Kempf et al., 2014). Microcephaly is caused by increased

apoptosis of RGCs and premature stem cell differentiation leading to altered cognitive performance (Jaylet et al., 2022; Otake and Schull, 1998; Wagle and Song, 2020). Additionally, several studies with exposure to IR on embryonic, young or adult rodents related the stimulation of neuroinflammation associated with neuronal loss and cognitive defects (Constanzo et al., 2020; Osman et al., 2020; Quintens et al., 2015; Verreet et al., 2016).

Effects resulting from ionizing radiation exposure of adults vary but microcephaly has never been detected. However a 4-year longitudinal study analyzing cognitive performance after Chernobyl accident on exposed individuals compared to healthy Ukrainians residing several hundred kilometres away from Chernobyl showed that performances were significantly lowered with decline in accuracy and efficiency and psychomotor slowing showing brain impairment (Gamache et al., 2005). Additionally, a linear association between the incidence of Parkinson's-disease and chronic exposure to gamma radiation was made on a cohort study of workers of a Russian nuclear production facility (Azizova et al., 2020). Studies in adult mouse confirmed the harmful effects of IR on mature brain. Indeed, Cs¹³⁷ radiation was shown to reduce mouse locomotor activity when exposed to 50 cGy or 200 cGy without affecting social exploration (York et al., 2012). Contrastingly, when 4-7 weeks old mice exposed prenatally were exposed to radiation from 0.10 Gy to 1 Gy, changes in sociability were detected (increase in nose contact) at both doses as well as a decrease in anxiety-related behavior (increased percentage of open arm entries and in ratio open/closed arm entries in the elevated plus-maze) at 1 Gy (Verreet et al., 2016).

According to UNSCEAR (2010 Report) the exposure levels corresponding to the domain of the dose and low dose rates are below 0.1 Gy or 6 mGy.h⁻¹ (UNSCEAR, 2010). In 2004, Hall and his team raised the possibility that even low dose rates of IR could be associated with decreased cognitive function in men who had received radiation for cutaneous haemangioma before age 18 months during 1930-59 (Hall et al., 2004). However, research in this area is still scarce and understanding the underlying mechanisms could help in predicting effects on cognition and behavior. Two transcriptional studies were performed on adult rodents to assess the molecular changes after exposure to 0.1 Gy. Overall, a major downregulation of genes involved in activities of neurotransmitters and their receptors (NMDA receptors), synaptic plasticity (genes involved in axon guidance, integrin signaling or synaptic long-term depression) or stress response (oxidative stress, Myc and Tp53) were detected (Lowe et al., 2009; Yin et al.,

2003). In zebrafish, ionizing radiation exposure mainly focused on reproduction with transgenerational effects or developmental processes (Hurem et al., 2017; Kamstra et al., 2018). Moreover, one study aimed at analyzing the modulation of genes after chronic exposure to IR on embryonic development with special attention on neurological effects. After radiating embryos at dose rates of 5 mGy.h⁻¹ and 50 mGy.h⁻¹, the main dysregulated pathways were related to cell cycle, synaptic transmission and neurogenesis where important developmental pathways were affected (Notch, retinoic acid, BMP and Wnt signaling pathways). They also observed a significant decrease of larval motility performing the light-dark locomotor test after exposing at 0.5 mGy.h⁻¹, 5 mGy.h⁻¹ and 50 mGy.h⁻¹ (Murat El Houdigui et al., 2019) .

Altogether, these studies unveil that ionizing radiation can modify gene signaling pathways involved in crucial functions such as neurogenesis or synaptic plasticity as well as muscle development in embryos specifically that can induce behavioral consequences, especially on learning and memory but also mobility, sociability and anxiety-related behavior. However, most of the studies aimed at deciphering the effects related to high dose of ionizing radiation or at the most radiosensitive stage of neurogenesis which is embryogenesis. In an ecological and even toxicological context, it is of great interest to understand the effects on chronic exposure to low dose of ionizing radiation on adult brain and assess how these modifications can alter behavior (for instance capacity to escape predators or to forage for food) and reproduction status. Herein, the continuous exposure of wild animals after a nuclear disaster, the increasing use of radiopharmaceuticals (i.e. medical diagnostics, radiotherapy) and the risks involving nuclear workers raise concern regarding health risks for both human and the environment.

1.3. Social and Stress behaviors

1.3.1. The social response

- **Generalities of the social response**

Social behavior can be defined as the interaction between individuals of the same species. This relation is essential for the survival and reproduction of social species. Diverse specific behaviors are related to sociability such as aggression, mating, conspecific preference or social communication. For example, honeybees exhibit brood

care or intricate dance language (Dolezal and Toth, 2014) and monk parakeets display monogamous pairs and constitute dominance hierarchies. Zebrafish are gregarious animals, they naturally form aggregated group while swimming which is a behavior called shoaling that appears as early as 15 days post fecundation and evolves with shoaling preferences during the juvenile phase (Engeszer et al., 2007; Hinz and de Polavieja, 2017). Shoaling is beneficial for an appropriate response for mating choice, detection and defence against predator resumed by the increase of shoal cohesion while foraging efficiency is characterized by decreased shoal cohesion (Oliveira, 2013). Various behavioral tests can be used for testing social behavior such as the individual social preference test (**Figure 14**). Classically, an adult fish is tested in a three-compartmented tank where a group of several fish (usually 2 males and 2 females) is swimming on one side of the tank. Time and number of entries of the tested fish near the shoal of conspecific is then determined to evaluate sociability. Diverse social stimuli can be used to assess preferences, for example between two different genetic strains (Geng and Peterson, 2019). Another type of test is to assess sociability within a shoal. The shoaling test is used in this case to assess the inter-individual and nearest neighbour distances between individuals within the shoal (**Figure 14**).

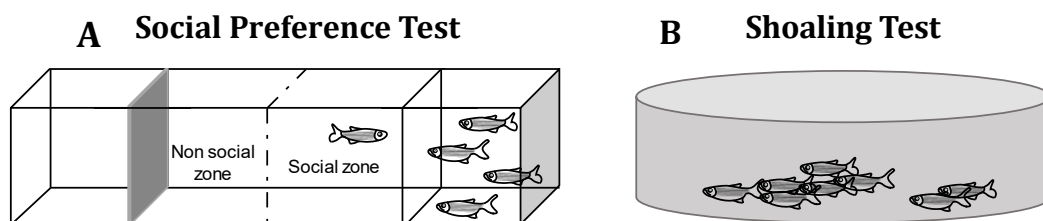


Figure 14 – Social response tested through behavioral experiments. In the social preference test (A), one testing fish is separated from a group of conspecifics (2 males, 2 females) so the intrinsic capacity of the fish to go interact with its conspecifics can be evaluated. We determine the number of crosses towards the social zone and the time that the testing fish stay in the social zone to consider a social fish or a non-social fish (most of the time in the non-social zone). In the shoaling test (B), a shoal of fish is disposed at the same time in a circular tank (in our case, 4 females and 4 males) and we evaluate the interindividual distances and nearest neighbour distances between the fish. The more the shoal is tight means that fish are stressed.

- **Neuroanatomy of the social response**

Based on neuroendocrine and behavioral studies, Newman, O'Connell and Hofman described a complex network of brain regions related to social behavior and highly conserved among vertebrates (Newman, 1999; O'Connell and Hofmann, 2011). This social decision-making network or SDMN gathers the social behavior network (SBN) and the basal forebrain/mesolimbic reward system. Each brain region is depicted as a node able to respond to diverse social stimuli and the interactions between nodes allow the regulation of behavioral outputs. In teleost, the SBN is composed of the ventral and anterior tuberal nuclei, the central gray and the preoptic area. Sex steroid receptors (e.g. estrogen receptor alpha, androgen receptors) is one signaling pathway these core nodes use to regulate social behavior (Forlano et al., 2010, 2005). The reward system contains the dorsal (Dm/Dl) and ventral telencephalon (Vd/Vc) and the posterior tuberculum, an area that was proposed by Wulliman to be homologous to the ventral tegmental area/substantia nigra which contains the dopaminergic system, central to this network (O'Connell and Hofmann, 2011). The involvement of the dopaminergic system in social reward was demonstrated in zebrafish with a direct correlation between the increase expression of dopamine in the brain and the exposure to conspecific images (Saif et al., 2013). Additionally, brain regions are shared between both systems, the supracommissural part of the ventral pallium (Vs) and the postcommissural nucleus of the ventral telencephalon (Vp) respectively corresponding in rodents to the medial amygdala (reward system in rodents) and to the nucleus of the stria terminalis and the ventral and lateral parts of the ventral telencephalon (Vv/Vl), the homologous of the lateral septum. Both nodes can be categorized as a crossroad for mediating information from a social stimulus converted to an adaptive behavior (ie. aggressivity toward intruder or sexual) (O'Connell and Hofmann, 2011). Suggested homologies with rodents, locations and connections between the different brain regions composing the SDMN are illustrated in **Table 2** and **Figure 15**. As mentioned before, these homologies need to be confirmed with further investigations. The connection between those systems (SBN and mesolimbic reward system) allows the evaluation of social stimuli, social learning and reinforce bonds and behaviors.

Table 2 – Putative homologies between brain regions in the social decision-making network of rodents and teleosts. From Ogawa and Parhar, 2022.

Classification of networks	Mammals (rodents) (Newman, 1999)	Teleosts (Goodson, 2005; O'Connell and Hofmann, 2011b, 2012)
Social decision-making network (SDMN)	Medial preoptic area (MPOA)	Preoptic area (POA)
	Anterior hypothalamus (AH)	Ventral tuberal nuclei (vTn)
	Ventromedial hypothalamus (VMH)	Anterior tuberal nuclei (aTn)
	Periaqueductal gray (PAG)	Central gray (GC)
	Medial amygdala (MeA)	Supracommissural part of the ventral pallidum (Vs) and
	Medial bed nucleus of the stria terminalis (mBNST)	Postcommissural nucleus of the ventral telencephalon (Vp)
	Lateral septum (LS)	Ventral and lateral parts of the ventral telencephalon (Vv and Vl)
	Ventral tegmental area (VTA)	Posterior tuberculum (nPT)
	Hippocampus (HPC)	Lateral part of the dorsal telencephalon (Dl)
	Basolateral amygdala (BLA or bLAMY)	Medial part of the dorsal telencephalon (Dm)
	Ventral pallidum (VP)	(unidentified)
	Nucleus accumbens (NAcc)	Dorsal and central parts of the ventral telencephalon (Vd and Vc)
	Striatum (Str)	
	Medial habenula (MHb)	Dorsal habenula (dHb)
	Lateral habenula (LHb)	Ventral habenula (vHb)

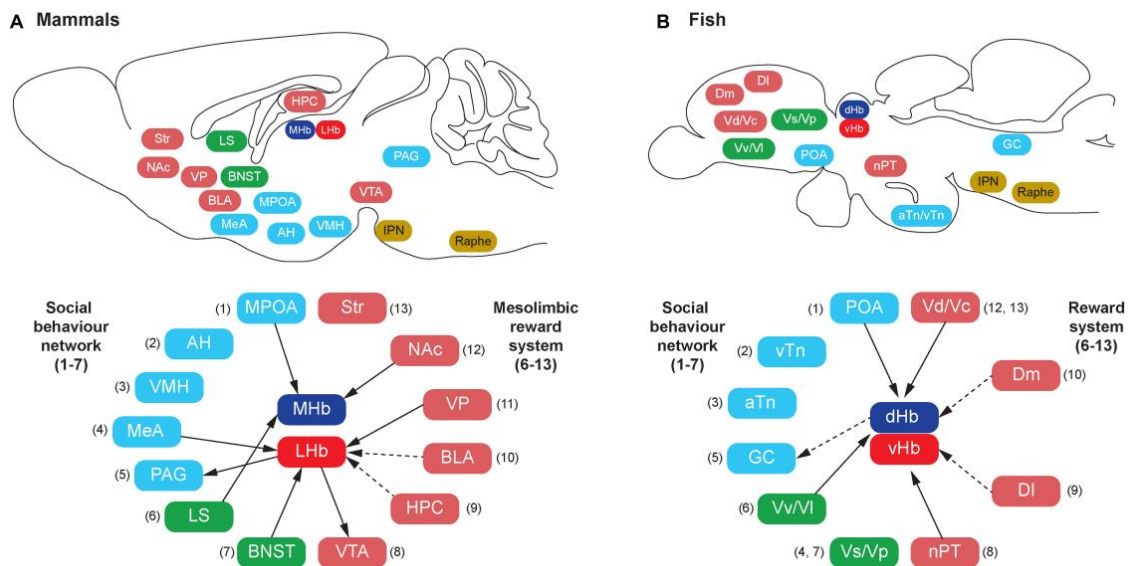


Figure 15 – Connections between habenula of mammals (A) and fish (B) and the social decision-making network. Abbreviations can be found in Table 2. From Ogawa and Parhar, 2022.

A recent functional study in zebrafish demonstrated that according to their social status (winners, losers, mirror-fighters), males present specific expression patterns and levels of immediate early gene across different nodes of the SDMN. For example, brain-derived neurotrophic factor (*bdnf*) plays a role in synaptic plasticity, neuronal differentiation 1 (*neurod1*) and wingless-type MMTV integration site family, member 3 (*wnt3*) act as indicators of neurogenesis and neuronal PAS domain protein 4a (*npas4*) enhances inhibitory synapses in response to excitatory transmission (Teles et al., 2016). The increase in neurogenesis genes (i.e. *neurod1*, *wnt3*) in Dm observed in Winners seems

specific to such individuals. Herein, depending on the social context, social animals need to adjust their behavior among conspecifics which relies on neuroplasticity of the SDMN (Teles et al., 2016). Understanding the involvement of each brain region in a specific social behavior task is consequently complex due to the many interactions between all the nodes shaping the SDMN. As an illustration, we will presently emphasize on the preoptic area (acting at the SBN level) and the role of hormones such as vasopressin and particularly oxytocin which is also involved in the stress response. Stress and sociability can be intricately together as autobiographical memories of intensively stressful experiences (e.g. post-traumatic stress disorder) can affect social and emotional behavior and cause social dysfunction, anxiety or depression in humans (Krammer et al., 2016; McMillan et al., 2014). For example, Meyer and colleagues demonstrated that memories encoded under stress conditions result in social deficits in male mice, but that dysfunction could be restored by inhibition of oxytocin receptor-positive interneurons localized in the hilus (Meyer et al., 2020).

- **The special case of the preoptic area and the role of oxytocin in social response**

In the rainbow trout, the preoptic area (POA) receives fibers from and projects to the telencephalon (e.g. Dd, Dl, Dm, Dc, Dp) and hypothalamus (Folgueira et al., 2004) and is subdivided in three regions established on cell size: the parvocellular region (homolog of the paraventricular nucleus of the mammalian POA), the magnocellular region and the gigantocellular region (homologue of the supraoptic nucleus of the mammalian POA). As in mammals, zebrafish oxytocinergic and vasopressinergic neurons are found in magnocellular and parvocellular neurons. Magnocellular cells are larger than the parvocellular ones, express higher levels of oxytocin, and project to other regions of the CNS and to the posterior pituitary for the release of oxytocin peptide into the bloodstream. Contrastingly, OXT parvocellular neurons can project to the hindbrain and spinal cord (Wee et al., 2019). The release of oxytocin/arginin vasopressin is ensured by axon terminals but also by dendrites to act locally (Ludwig, 1998; Onaka et al., 2012). Using *in vitro* and *in vivo* mice mutants, the transcription factors aryl hydrocarbon receptor nuclear translocator 2 (ARNT2) and single-minded 1 (SIM1) were demonstrated to be involved in the differentiation of oxt neurons (Michaud et al., 2000, 1998). In *Danio rerio*, oxytocin can bind to two receptors, originated from the gene duplication event, the oxytocin receptor-like *oxtrl* and the oxytocin receptor *oxtr*. For its part, arginin

vasopressin has three receptor subtypes: vasopressin receptor AVPR1A, AVPR1B and AVPR2, with involvement for example in stress adaptation, aggressive behavior, social memory and other social behaviors (e.g. pair bonding) in mammalian species and in teleost there appear to be two V1a receptors (Avpr1aa and Avpr1ab) and three V2 receptors (Avpr2aa, Avpr2ab, Avpr2l) (Braida et al., 2012; Caldwell, 2017; Iwasaki et al., 2013; Larson et al., 2006). The structure of both receptors (oxytocin and arginine vasopressin) is very similar thereby the actions of the neuropeptides may overlap which can complexify the study of its respective involvement in stress and social behaviors (Neumann and Landgraf, 2012). Beside oxytocin, arginin vasopressin was demonstrated to play a role in aggression and in reducing social interaction but not shoaling behavior (Lindeyer et al., 2015). For a long time, oxytocin was only considered for its role in lactation and facilitator of childbirth in mammals. However, in the 1970s, Oxt was shown to play a role on social behavior by Pederson and Prange from the induced maternal behavior after intracerebroventricular injections of oxytocin in virgin female rats (Pedersen and Prange Jr, 1979). In adult zebrafish, the use of pharmacological approaches with administration of exogenous Oxt, demonstrated the increase of social preference and decrease of fear response in a dose-dependent manner (Braida et al., 2012). Additionally, the administration of the Oxt agonist carbetocin showed a rescue of social deficits when adult zebrafish were treated by a glutamate antagonist (Zimmermann et al., 2016). The ablation of Oxt neurons of the posterior tuberculum affected social preference in 6-day-old larvae. In zebrafish, the exact role of oxytocin receptors in sociability is still controversial, particularly on social preference. Indeed, when knocking down the receptor *oxtr*, only an effect on social recognition by measuring the ability to discriminate between novel and familiar fish was noticed without affecting social preference indicating here that OXTR is not directly involved in the motivation that drive to approach conspecifics (Ribeiro et al., 2020). However, the receptor *Oxtr1* was proposed to play a redundant role and therefore could rescue the social preference response after *oxtr* knock out. Landin and his team evaluated the effect of the nonpeptidergic oxytocin receptor antagonist L-368,899 which target both OXT receptors and concluded in a decreased social preference in larval and adult's zebrafish (Landin et al., 2020). Additionally, after knocking down *oxtr* or *oxtr1* paralleled with isolation of 2-to-8 weeks old fish, the maintenance of social preference and shoaling behavior was modified (Gemmer et al., 2022). Altogether it seems that both receptors don't play the same role with a prominent role of *oxtr* in social recognition and *oxtr1* in social preference in adult zebrafish, and that

both the age and the social context can influence receptors signaling to regulate social preference by oxytocin. It is also worth to mention that the neurotransmitters serotonin and dopamine can interact with oxytocin in the regulation of social behavior (**Figure 16**) (Onaka et al., 2012; Teles et al., 2013) and fish treated with a dopamine D1 receptor antagonist decreased social preference behavior (Scerbina et al., 2012). Even if the coordinated activity of oxytocin and serotonin was mainly studied focusing on the anxiolytic role of oxytocin through oxtr-serotonergic neurons in mice (Yoshida et al., 2009), Dölen and colleagues demonstrated that coordination between serotonin and oxytocin is involved in the rewarding properties of social interaction in mice (Dölen et al., 2013). However, these interactions were not demonstrated in zebrafish. Finally, the role of oxytocin is not restricted to social behavior in both mice and zebrafish. Indeed, the neuropeptide was demonstrated to regulate food intake and energy balance with capacity to induce anorexigenic effects, produce depression-like forced swimming behavior, reduce avoidance behavior during shock training or anxiety in the open field test in rodents (Chaviaras et al., 2010; Stoehr et al., 1992; Takayanagi et al., 2005; Yoshida et al., 2009).

1.3.2. The stress response

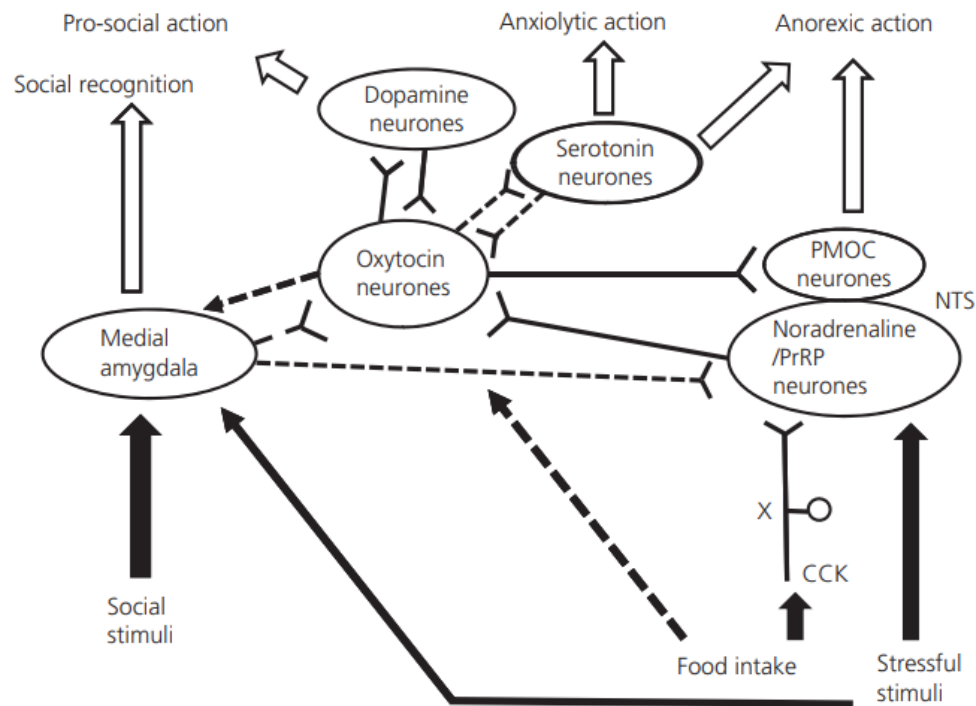


Figure 16 – Possible oxytocin pathways regarding stress, energy metabolism and social behavior in mammals. Food intake and stressful stimuli activate hypothalamic oxytocin neurons via noradrenaline. prolactin-releasing peptide neurons in the nucleus tractus solitarius. For social stimuli, the activation of oxytocin neurons involves the medial amygdala. Anxiolytic, anorexic or pro-social actions are activated through serotonergic neurons in the median raphe nucleus, proopiomelanocortin neurons in the nucleus tractus solitarius and the medial amygdala respectively. Oxytocin-dopamine neurons may be involved in the rewarding aspects of social preference. These interactions have not been demonstrated in the zebrafish yet. CCK: Cholecystkinin, NTS: Nucleus tractus solitarius, POMC: Proopiomelanocortin, PrRP: prolactin-releasing peptide neurons, X: Vagus nerve. From Onaka et al., 2012.

- **Generalities of stress behaviors**

Stressors, either being intrinsic (e.g. pregnancy, lactation, molting) or extrinsic stimuli (e.g. pathogens, resource limitation temperature), are considered as a threat that can affect animal organisms by disturbing their homeostasis. The physiological response act as a compensatory system in order to overcome the threat. The stress response is thus an acclimation process that aim at coping with the altered environment. In some cases, the acclimated response is not sufficient or might lead to costs that can result in inhibition of growth, reproductive failure or reduced resistance to pathogens (Wendelaar Bonga, 1997). In humans, the difference between stress and anxiety mainly relies on that stress

is activated by external factors and is a normal bodily response cued by the brain and nervous system while anxiety is a psychological condition that can develop when stress response occurs too often or is too long. Here, since we cannot have access to a “psychological” response of zebrafish we will consider the terms as similar. Multiple mechanisms can be affected by stress such as the deregulation of neurotransmitters and hormones or the modulation of behaviors (de Abreu et al., 2021). In zebrafish, investigation of fear and anxiety can be evaluated through several behavioral tests. For example, larval zebrafish can trigger an escape behavior by detecting through their lateral line the water movement of a predator. To specifically test anxiety, various assays have been established such as is the novel tank test, considered as one of the most direct measures of anxiety in adult zebrafish (**Figure 17A**). This assay evaluates the time spend in the bottom of the tank, erratic movements and freezing which are indicators of the stress response (Clark et al., 2011). Another test is the preference for dark over light environments (Jesuthasan, 2012) (**Figure 17B**).

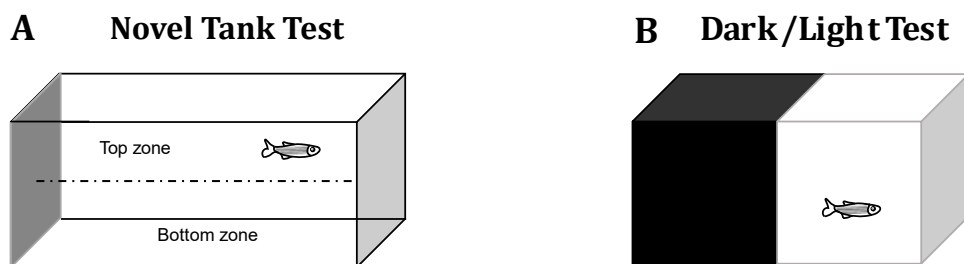


Figure 17 - Stress responses tested through behavioral experiments. In the novel tank test (A) the testing fish is introduced in a narrow tank where we evaluate the time spent in the top/bottom zone and number of entries in each zone. The more the fish spend time in the bottom zone generally means that the fish is in a stress state. The Dark/light test (B) measures the dark avoidance behaviour of fish when they are in a stress state.

- **Neuroanatomy of the stress response- involvement of the HPI axis**

In zebrafish, the stress response is mainly mediated via the hypothalamic pituitary-interrenal axis (HPI axis), the homologous of the mammalian hypothalamic-pituitary adrenal axis. The HPI axis is composed of the hypothalamus, especially the nucleus preopticus or neurosecretory preoptic area (homologous of the paraventricular nucleus PVN) where stress stimulus will activate the release of corticotropin releasing factor (Crf or Crh) inducing in the anterior pituitary the secretion of proopiomelanocortin (Pomc) which is the precursor of Adrenocorticotrophic hormone (Acth). Finally, Acth will stimulate the release of cortisol by the interrenal tissue. Two receptors Crfr1 and Crfr2 were

characterized in fish (e.g. in carp) but Crfr1 is the one that mediates the stress response (Huisin et al., 2004). In zebrafish, two receptors can be described: Crhr1 and Crhr2. Acth acts on melanocortin 2 receptor (Mc2r) to stimulate cortisol synthesis that can bind to mineralocorticoid receptors and to glucocorticoid receptors. In mammals, ligand binding acts to restore homeostasis by producing negative feedback to HPA axis (Eachus et al., 2021). This negative feedback is mediated through type II glucocorticoid receptors (Charmandari et al., 2005) and is essential to minimize the catabolic, lipogenic, anti-reproductive and immunosuppressive actions of glucocorticoids (Charmandari et al., 2005).

- **Other modulators of the stress response**

Additionally to cortisol, other mechanisms are involved in the regulation of vertebrates including zebrafish stress response with similar neurochemical and neuroendocrine mechanisms to those in mammals (Schreck and Tort, 2016; Wendelaar Bonga, 1997). For example, catecholamines (epinephrin and norepinephrine from the hypothalamic-sympathetic-chromaffin cell axis) are released almost immediately after stress exposure, earlier than Crf (Demin et al., 2021). Moreover, an interaction between the serotonergic system and the hypothalamic stress axis was proposed in mammals (Lanfumeu et al., 2008). Indeed, the introduction of 5HT receptor ligands can stimulate ACTH and cortisol plasma secretion in mammals and humans (Klaassen et al., 2002; Wetzler et al., 1996). Reciprocally, in rat, it was demonstrated that CRH can activate a subpopulation of serotonergic neurons in the raphe nuclei which in turn mediates anxiety-like behavior (Meloni et al., 2008). In zebrafish, fluoxetine treatment which is a selective serotonin reuptake inhibitor increases glucocorticoid receptor expression (Erdeljan et al., 2001) and reduces whole-body cortisol levels. Also, this treatment can normalize the affected stress response behavior (freezing and reduced exploration) in mutant grs357 zebrafish (Griffiths et al., 2012).

In addition, melatonin was shown to be involved in acute and chronic stress responses in many species, including in fish. Indeed, net chasing and high stocking density inhibited melatonin synthesis (Genario et al., 2019; Kopp et al., 1999) in the pineal gland of rainbow trout and the administration of melatonin in zebrafish was able to reduce anxiety-like behaviors (Genario et al., 2019) demonstrating a conserved role with mammals.

Oxytocin neurons can be activated by norepinephrine/prolactin-releasing peptide (PrRP) neurons in the nucleus tractus solitarius in response to a stress stimulus in

mammals (Onaka et al., 2012). This neuropeptide was mainly described as a facilitator of an anxiolytic response. For example, in ovariectomized estradiol-treated female rats exposed to white noise stress (114 dB for 10 min) showed that when they are supplemented with central infusions of oxytocin, the response to the stress stimulus was dose dependently decreased with less rearing behavior, a higher proportion and more time spent in open arm entries on the elevated plus-maze (Windle et al., 1997). Even if controversies still exist, several hypotheses were proposed regarding its anxiolytic actions.

❖ Involvement of serotonin

First, the fact that oxytocin injection induced serotonin release via serotonergic neurons expressing oxytocin receptors and secondly, that serotonin 2A/2C receptor antagonist can impair anxiolytic actions of OXT, suggested that OXT can decrease anxiety by the release of serotonin (Onaka et al., 2012).

❖ Involvement of medial amygdala

Also, the amygdala was proposed to be targeted by oxytocin in the regulation of the fear response. Indeed, after inhibition of central amygdala, which caused conditioned fear stimuli, oxytocin could decrease freezing behavior via activation of GABAergic neurons. Another illustration is the administration of an oxytocin receptor antagonist into the central amygdala that increased anxiety-related behavior in late pregnant rats (Onaka et al., 2012).

❖ Involvement of CRF

An added explanation is the direct and indirect effect of oxytocin on CRF. It was demonstrated that oxytocin can regulate *Crf* gene transcription by modulating the nuclear translocation of the co-activator CRTF3 and this might delay stress-induced CRF expression in the PVN of male rats, mice and human neuronal cells (Jurek et al., 2015). GABAergic neurons positive to OXTR were shown to inhibit CRF neuronal activity, demonstrating here the indirect effect of OXT (Winter and Jurek, 2019). Also, the local administration of an antagonist of oxytocin receptor in male rats was demonstrated to facilitate ACTH responses after a stressful stimulus (Onaka et al., 2012). Herein, more studies are needed in order to discriminate the exact action of oxytocin on the regulation of the HPA axis.

Regarding studies on zebrafish, after environmental stressors such as acidic and high ammonia, an alteration (increase or decrease) of oxytocin receptors in the brain was observed and complemented by a decreased of the anxiety level in high ammonia treated fish. Moreover, the number of oxytocin neurons was increased after double-deionized treatment. Hereafter, oxytocin signaling was proposed as a stress indicator upon environmental stressors (Chuang et al., 2021). Additionally, axons of oxytocin were found to project into the interpeduncular nucleus (IPN) and raphe of adult brain supplemented by a downregulation of aggressiveness. In fact, IPN is connected to the habenula well known for its function in aggression or fear response and habenula was shown to take part in the regulation of anxiety and social behavior (Chuang et al., 2021).

1.3.3. A possible crossroad between social and stress responses: the habenula

In zebrafish, two subregions constitute the habenula: the left-directed dorsal asymmetric habenula (different volumes of nuclei) and the ventral symmetric habenula. The dorsal habenula is subdivided into lateral and medial subnuclei. The afferent connections of the habenula with nodes of the SDMN were partially described in zebrafish, such as connectivity to the Vv, Vd, the POA or the nPT. The lateral subnuclei of the dorsal habenula (dHbl) and the medial subnuclei of the dorsal habenula (dHnM) project to the dorsal and ventral part of the IPN respectively. The dIPN then connect to the dorsal part of the superior raphe to terminate at the central gray while the vIPN projects to the median raphe. The vHb terminates to the ventro-anterior area of the median raphe (Okamoto et al., 2012) (**Figure 18**).

Disruption of the neural signal transmission from the lateral subnucleus of the dorsal habenula to the dorsal IPN induced a freezing response when fish were exposed to successive conditioned adverse stimuli in comparison to control animals that developed avoidance behavior. This data led to the proposal that the dorsal-habenula-IPN pathway plays a role in reducing anxiety response (Okamoto et al., 2012). In addition, another study that ablated medial habenula (via tetanus toxin) showed that fish may present a higher stress baseline based on the novel tank test (Mathuru and Jesuthasan, 2013).

Regarding the ventral habenula, it was recently demonstrated to play a role in social decision making related to fear and anxiety in zebrafish (Okamoto et al., 2021). For example, in the chronically presence of aversive cues, activation of serotonergic neurons of the median raphe nuclei through the activation of ventral habenula neurons in

zebrafish suggested that this vHb-serotonin pathway is involved in the social risk evaluation (expectation of a social threat) (Amo et al., 2014; Ogawa and Parhar, 2022).

Recently, the habenula was found to play a role in social and reproductive behaviors. In rats, it is both involved in the positive response of social response (rewarding effect) like the onset of maternal behavior and as already described, in the negative aspect (stressful effect) such as anxiety and aggression. Additionally in fish, the dorsal habenula was established to regulate social fighting: in social conflict situations, the dHbl-dIPN pathway is activated when fish are considered as “winners” however the dHbl-vIPN is activated in “loser” fish (Cherng et al., 2020). Since, the habenula is involved in regulating monoaminergic systems (dopamine and serotonin) it is therefore interconnected between various network, the SDMN and the stress response system. It was demonstrated that in mammals as in fish, neuropeptidergic neurons projects to the habenula which expresses neuropeptides receptors, strongly suggesting the involvement of habenula in regulating social and anxiety behaviors (Ogawa and Parhar, 2022).

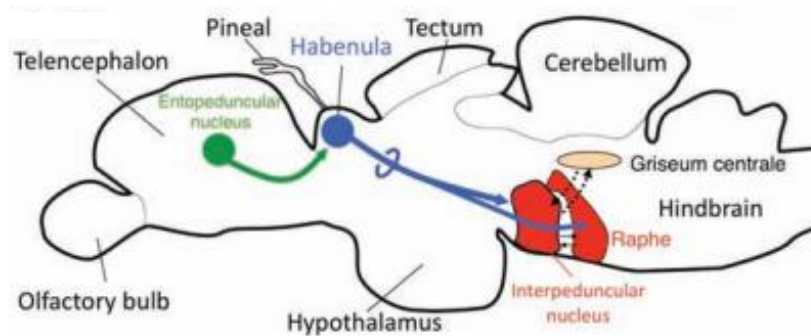


Figure 18 – Afferent and efferent neural pathways of the habenula. From Okamoto et al., 2012.

2. Research axes and methodology

2.1. Research axes

The main objective of this project was to decipher what are the effects of the radiation-induced effects on the brain of adult zebrafish. Thus, two research axes ensued from that perspective. The first one aimed at characterizing the molecular, cellular and behavioral effects of chronic low to moderate dose rates of ionizing radiation by a multiscale approach. The second axis focused on comparing the molecular events occurring after two different types of stressors, the one caused by ionizing radiation and another one, the physical injury of the brain by wounding. This comparison aimed at understanding the effects on the process of neurogenesis since it is well known that stabwound injury induces reactive neurogenesis dependent on neuroinflammation.

2.1.1. Axis I: From molecular effects to stress behavior and reduced sociability

To address this topic, several questions were brought to light:

Question 1: What are the main molecular modifications after chronic low to moderate dose rates of ionizing radiation?

We want to compare various dose rates and hypothesize that the highest dose rate would induce more effects. We are also interested in knowing if we could detect a threshold dose rate under which no molecular effect can be detected. Finally, we hypothesized that the main molecular pathways affected by chronic exposures will be different from the ones observed after high doses and acute exposures to radiations.

Question 2: Is there any modification at the organism scale and could it be predicted by the transcriptomic analysis?

Herein, axis I was completed by a behavioral analysis to first decipher the consequences of chronic low to moderate IR at a higher scale. The objective here was to detect any modification of behavior that could affect sociability, stress and locomotion with potential consequences on reproduction, efficacy of foraging and survival. Trying to

link the molecular modification with an impact on behavior could also allow to propose specific responses to chronic low to moderate dose rates of IR and anticipate an appropriate response in the framework of the improvement of risk assessment of IR in ecotoxicology.

2.1.2. Axis II: Common and differential features of IR response compared to regenerative neurogenesis induced by neuroinflammation

Many studies, specifically in rodents, showed that acute and high doses of ionizing radiation could cause the reduction of neurogenesis. As induction of neurogenesis is observed after stabwound injury of the brain in zebrafish, we investigated if the mechanisms caused by chronic low to moderate dose rates of ionizing radiation were similar to those occurring during neuroregeneration. Thus, here are the following questions brought up under this axis:

Question 1: What can we learn from transcriptomic analyses after wounding adult zebrafish telencephalon?

Few transcriptional analyses have already been performed after stabwound injury in adult zebrafish telencephalon. These studies mainly focused on specific effects at distinct times after injury. We investigated here the mechanism of brain regeneration in a holistic way, including a study from early effects (1 hour and 3 hours post stabwound injury) until the resolution of the wound (10 days after lesion).

Question 2: What are the specificities of radiation induced effects in the brain?

Comparing transcriptomic analyses between two sorts of stressors might help us to clarify the specific pathways affected after chronic low to moderate dose rates of IR. This could help us propose new molecular markers of the effects of chronic exposures to IR. Additionally, we wanted to test whether initiation of a regenerative neurogenesis after exposure to IR (observed in axis I) was dependent on neuroinflammation.

2.2. Global experimental design

Effects of ionizing radiation were evaluated on adult wildtype AB zebrafish, from 6-to-9 months old. Fish were exposed in the platform MICADO'Lab (Moyen d'Irradiation Chronique pour l'Acquisition de relations Dose effet en Laboratoire) located in Cadarache (IRSN) (Figure 19).



Figure 19 – Irradiation Hall – MICADO'Lab with 4 sources (orange boxes) and fish racks.

This platform is dedicated to chronic external gamma radiation exposure and is composed of four sources of cesium 137 with total activity of 370 GBq. The range of dose rates can vary from 0.005 mGy.h^{-1} to 100 mGy.h^{-1} depending on the distance to the source. Before experimental radiation, we modelled the dose rate in the aquarium tank in order to calculate the distance of organism related to the source as follow:

- We must consider the attenuation of ionizing radiation that cross water. This was performed by modelling the exposure of tanks with the Monte-Carlo N-Particle transport code (MCNP) (performed by Nicolas Dubourg)

- 2 Radio Photo Luminescents (RPL) were placed on each tank to confirm the target dose rate: one in front and one at the back of the tank to measure dose rates and compare them to the modeled dose rates (performed by Nicolas Dubourg).

We selected three dose rates for continuous exposure for 36 days: 0.05 mGy.h⁻¹, 0.5 mGy.h⁻¹ and 5 mGy.h⁻¹. Those exposure levels correspond to the domain of low dose rates (<6 mGy.h⁻¹ (UNSCEAR, 2010), the lower dose rate is close to the reference value for ecosystem radioprotection (0.010 mGy.h⁻¹) (Andersson et al., 2009) and the middle dose rate correspond to fish exposed in Chernobyl after the accident (estimated at 0.4 mGy.h⁻¹) (Lerebours et al., 2018).

In (eco)toxicology, molecular, biochemistry, cellular or physiological modifications are measured in the organism and if predictive of effects on mortality, growth and reproduction, are called biomarkers. However, the detection of a biomarker cannot reveal the exact mode of action of contaminants. Herein, toxicogenomics has considerably developed recently and allows the detection and comprehension of biological responses earlier than individual endpoints. In addition, toxicogenomics propose an holistic understanding of the effects by analyzing all molecules (in our case transcripts) altered by stressors, providing thus an unbiased analysis of the effects compared to biomarkers (which are used as proxy of known effects). In this project, we applied an approach that coupled toxicogenomics studies with effects at higher biological scales, such as behavioral analysis, to decipher the underlying mechanisms and possibly propose links between these different scales. Thereafter, we proposed a global analysis through transcriptomics to first have an overview of the main modulations on gene expression after radiation. We also compare those results to the transcriptomics of another type of wounding which is the stabwound injury in order to better apprehend the underlying mechanisms involved. We conducted various stabwound injuries to apprehend the molecular events at 1 hour post lesion (hpl), 3 hpl, 10 hpl, 24 hpl, 96 hpl and 240 hpl. Additionally, we used *in situ* RNA hybridization on relevant candidates to benefit from valuable information on spatial identity on brain sections. Finally, we performed behavioral analyses on irradiated fish and established 3 tests: the novel tank test, the social preference test and the shoaling test.

3. Results & Discussion

3.1. Axis I: From molecular effects to stress behavior and reduced sociability

3.1.1. Objectives of scientific paper

The nuclear accidents of Chernobyl (1986) or Fukushima Daiichi (2011) released high levels of ionizing radiation (IR) in the environment and high levels of IR are known to induce neurogenesis defects with harmful consequences on brain morphogenesis and cognitive functions. However, the effects after chronic low to moderate dose rates of IR are well less characterized which brought us to the framework of this paper. We aimed at understanding the main molecular pathways affected through a global analysis by performing transcriptomics. We completed this analysis by *in situ* RNA hybridization to confirm the previous results observed. Finally, to decipher the consequences on the organism scale, we performed behavioral tests.

3.1.2. Revealing the increased stress response behavior through transcriptomic analysis of adult zebrafish brain after chronic low to moderate dose rates of ionizing radiation

This article was submitted the 25th of June 2022, accepted the 1st of August 2022 and published the 4th of August 2022 in the journal “MDPI-Cancers” under the special issue “Cancer and non-cancer effects following Ionizing Irradiation”. [Cancers | Free Full-Text | Revealing the Increased Stress Response Behavior through Transcriptomic Analysis of Adult Zebrafish Brain after Chronic Low to Moderate Dose Rates of Ionizing Radiation \(mdpi.com\)](#)



Article

Revealing the Increased Stress Response Behavior through Transcriptomic Analysis of Adult Zebrafish Brain after Chronic Low to Moderate Dose Rates of Ionizing Radiation

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Citation: Cantabella, E.; Camilleri, V.; Cavalie, I.; Dubourg, N.; Gagnaire, B.; Charlier, T.D.; Adam-Guillermin, C.; Cousin, X.; Armant, O. Revealing the Increased Stress Response Behavior through Transcriptomic Analysis of Adult Zebrafish Brain after Chronic Low to Moderate Dose Rates of Ionizing Radiation. *Cancers* **2022**, *14*, 3793. <https://doi.org/10.3390/cancers14153793>

Academic Editor: Nobuyuki Hamada

Received: 25 June 2022

Accepted: 1 August 2022

Published: 4 August 2022

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Simple Summary: The increasing use of radiopharmaceuticals for medical diagnostics and radiotherapy raises concerns regarding health risks for both humans and the environment. Additionally, in the context of major nuclear accidents like in Chernobyl and Fukushima, very little is known about the effects of chronic exposure to low and moderate dose rates of ionizing radiation (IR). Many studies demonstrated the sensibility of the developmental brain, but little data exists for IR at low dose rates and their impact on adults. In this study, we characterized the molecular mechanisms that orchestrate stress behavior caused by chronic exposure to low to moderate dose rates of IR using the adult zebrafish model. We observed the establishment of a congruent stress response at both the molecular and individual levels.

Abstract: High levels of ionizing radiation (IR) are known to induce neurogenesis defects with harmful consequences on brain morphogenesis and cognitive functions, but the effects of chronic low to moderate dose rates of IR remain largely unknown. In this study, we aim at defining the main molecular pathways impacted by IR and how these effects can translate to higher organizational levels such as behavior. Adult zebrafish were exposed to gamma radiation for 36 days at 0.05 mGy/h, 0.5 mGy/h and 5 mGy/h. RNA sequencing was performed on the telencephalon and completed by RNA in situ hybridization that confirmed the upregulation of oxytocin and cone rod homeobox in the parvocellular preoptic nucleus. A dose rate-dependent increase in differentially expressed genes (DEG) was observed with 27 DEG at 0.05 mGy/h, 200 DEG at 0.5 mGy/h and 530 DEG at 5 mGy/h. Genes involved in neurotransmission, neurohormones and hypothalamic-pituitary-interrenal axis functions were specifically affected, strongly suggesting their involvement in the stress response behavior observed after exposure to dose rates superior or equal to 0.5 mGy/h. At the individual scale, hypolocomotion, increased freezing and social stress were detected. Together, these data highlight the intricate interaction between neurohormones (and particularly oxytocin), neurotransmission and neurogenesis in response to chronic exposure to IR and the establishment of anxiety-like behavior.

Keywords: gamma irradiation; teleost; anxiety-like behavior; sociability; neurotransmitter system; neurohormone

1. Introduction

Ionizing radiation (IR) causes oxidative stress and genotoxic damages that can interfere with cellular activity and, directly or indirectly, with biological functions. Among their deleterious effects, cancer was originally found to be one of the main risks associated with exposure to IR [1–3]. In addition, epidemiological data on Japanese atomic bomb survivors (Hiroshima and Nagasaki in 1945) also demonstrated an elevated risk of developmental neurological disorders, especially microcephaly, in the prenatally exposed population [4]. Similar observations were taken in wild species exposed to IR due to nuclear power plant (NPP) accidents. After Chernobyl (1986) or Fukushima Daiichi (2011), yearling birds and *macaca fuscata* fetuses from contaminated areas displayed decreased brain size [5,6]. However, the majority of the studies described in the literature covers high and acute dose rates of IR (>6 mGy/h, [7]), and the biological effects of low dose rate radiation remains a subject of broad societal and scientific questioning and concern [8]. The impact of chronic exposure to low to moderate dose rates of IR on adult brain functions remains largely unknown and their impact on behavior and reproduction or survival is currently little studied [9].

Brain development is particularly sensitive to IR and other stressors (infections, ischemia, toxicants) [10,11]. Prenatal exposure in rodents to high doses of IR (more than 1 Gy) or X-rays reduces the number of neurons [12,13], decreases the complexity of dendritic arborization [14] as well as the number of neuronal progenitors after adults' exposure [15] and can finally result into behavioral and memory defects [16,17]. In addition, recent work in humans demonstrates that IR can also alter brain homeostasis and function. Indeed, prenatal exposure to IR was suggested to impact cognitive functions with reduced school test results, lower verbal IQ or lower logical reasoning in adolescents [18–20]. However, the impact of IR is not restricted to the development period and was also proposed in adults with a decline in the accuracy and efficiency of cognitive performance [21,22]. Moreover, the effects of IR on adult neural stem cells or proliferating neural precursor cells were also demonstrated in the subventricular zone and the hippocampus of rodents [23–28]. In the context of chronic exposure to low dose rates of IR, fewer data exist on the effects on the central nervous system. For instance, an elevated risk of Parkinson's disease was observed in a cohort of nuclear Russian workers which was strongly suggested to be linked with chronic exposure to external γ -rays and neutrons [29]. Moreover, studies in mice demonstrated that exposure to a much lower dose (0.1 Gy) could lead to the downregulation of neurotransmitters and genes with functions in synapses and neuron projections [30,31]. These results support the notion that the cellular response to chronic low dose and high dose and dose rates are different and further describe the emerging evidence of the neurological effects of exposure to radiation at dose rates much lower than previously thought in humans [29,32–34].

Stress response and anxiety-like behaviors are commonly quantified to assess the effects of toxicants on brain function [35,36]. In response to environmental stress, the adrenocorticotrophic hormone (ACTH) is released by the pituitary gland and acts on the adrenal gland to stimulate the synthesis and secretion of glucocorticoids, which will in turn orchestrate the stress response via cortisol release [37]. This neuroendocrine system, denominated as the hypothalamic–pituitary–adrenal (HPA) axis, is conserved in mammals and fish (given that the interrenal organ in teleost is the homologue of the adrenal gland) and controls the stress response. The peripheral stress response induced by ACTH is controlled centrally by the release of the corticotropin release factor (CRF). In addition, other hypothalamic neurohormones such as oxytocin or arginine vasopressin also play a role in the regulation of the stress response. Indeed, the ablation of oxytocin expression in knockout mice induced anxiety-like behavior and increased the corticosterone level in the blood after the psychogenic stressor [38]. Additionally, stress elicited by forced swimming induced oxytocin release in the hypothalamus and peripheral circulation in rodents [39], which thus demonstrated an intricate interaction between oxytocin and the stress system. Moreover, in rodents, oxytocin can interact with the dopaminergic system or the serotonergic

system to influence a social response and to regulate anxiolytic effects, especially via the 5-HT_{1A} and 5-HT_{2A/C} receptors [40–47]. In zebrafish, somas of oxytocin neurons are found in the parvocellular preoptic nucleus corresponding to the paraventricular nucleus of the hypothalamus in rodents and in the periventricular nucleus of the posterior tuberculum [48–50]. As in mammals, oxytocin was also demonstrated to play a role in sociability in the zebrafish, notably in the development and maintenance of social behavior through oxytocin receptors (oxtr and oxtrl) [49,51]. Consequently, modifications in behavior could impact foraging and predator avoidance and interfere with reproduction.

With its simple cortical organization and an abundant constitutive neurogenesis, the zebrafish model, *Danio rerio*, is of great interest to study molecular mechanisms and behavior [52,53]. Moreover, this model accommodates easily to laboratory conditions and demonstrates a high degree of sociability thus making zebrafish a robust model for neuroscience research.

In this study, we aimed at characterizing the effects of IR impact on stress and behavior using adult zebrafish as a model system. The fish were exposed to dose rates of 0.05 mGy/h, 0.5 mGy/h and 5 mGy/h for 36 days. Those exposure levels correspond to the domain of the low dose rates (<6 mGy/h, [7]), are near the reference value for ecosystem radioprotection (0.010 mGy/h) [54] and are in the range of the dose rates estimated after the Chernobyl accident (0.4 mGy/h) [55]. A multiscale approach was used to evaluate the effects on adult behavior, with emphasis on sociability, stress and locomotion. The molecular effects of chronic exposure to IR on the brain were assessed through a transcriptomic approach, completed by the study of spatial changes in gene expression of *oxytocin* and *cone rod homeobox* by RNA in situ hybridization.

2. Materials and Methods

1.1. Zebrafish Maintenance and Irradiation

Adult zebrafish were maintained at the Institut de Radioprotection et de Sûreté Nucléaire facilities in accordance with French and European regulations on the protection of animals used for scientific purposes (EC Directive 2010/63/EU and French Decret 2013–118). All experiments were approved by the ethical comity (C2EA, IRSN, Fontenay-aux-Roses, France) and the French Ministry of Research under the reference APAFIS#11488. Six- to nine-month-old wildtype zebrafish of the AB strain were provided by Amagen (Gif-sur-Yvette, France) and Sorbonne Paris University (Institut de Biologie Paris Seine, Paris, France). Constant conditions were preserved for fish maintenance: 27 ± °C, 350–450 µS/cm, pH 7.5 and 12/12 h dark-light cycles, in the ZebTEC system (Techniplast, Décines-Charpieu, France). The fish were fed three times a day with Gemma Micro 600.

Any mortality was noted, the homogeneity of the fish's weight and length were verified after behavior experiments, and no difference was observed in any condition or between males and females (data not shown). The fish were chronically exposed for 36 days to gamma irradiation by a ¹³⁷Cs source in the MICADO experimental irradiation facility (IRSN, Cadarache, Saint-Paul-lez-Durance, France), except for a duration of one hour five times a week without irradiation to visually verify the fish's health. Three dose rates were employed: 0.05 mGy/h, 0.5 mGy/h and 5 mGy/h. To confirm the dose rates of exposure, we used operational dosimetry with radio-photoluminescent dosimeters (RPL, GD-301 type, Chiyoda Technol Corporation Japan, Tokyo, Japan), and the effective dose rates were estimated by MCNPX v2.70 (Supplementary Materials, Table S1).

1.2. Behavioral Analysis

1.2.1. Novel Tank and Social Preference Tests

The Novel Tank Test (NTT) was used for the assessment of the stress behavior and locomotor profiles of adult zebrafish. Single fish ($n = 20$ females and males per condition) were placed for 6 min in a tank (27.9 x 7.1 x 15.2 cm) filled with water (13.5 cm deep).

Videos were recorded from the side using a Basler acA1300–60 gm camera fitted with an infrared filter detection (Infrarot 850, Heliopan, Noldus Information Technology, Wageningen, Holland) and a bespoke infrared spotlight platform to avoid shadows and artefacts. To study the locomotion, we measured the total distance travelled. For behavioral parameters related to stress, we designed an arena in Ethovision XT14 that separated the tank into top and bottom zones of equal dimensions and measured the following parameters: time spent and number of entries in top zone, latency to first entry to the top zone and immobility time. Mobility was defined as the degree of the displacement of the zebrafish's body independent of spatial movement of the center body point [56].

The social preference test was performed as previously described in order to assess how the fish interacts towards a social stimulus [57,58]. One minute after the end of the NTT, this trial was performed for a total of 5 min using the same tank as for the NTT to avoid stress, except that a new compartment of 15 x 7.1 x 15.2 cm with four stimulus fish (two females, two males) was located on one side of the central arena. We divided the central arena that includes the test fish into two equal parts (non-social and social zones). An additional zone measuring half the body length of the fish (Z1) was included in the social zone close to stimulus fish (Figure 2). The size of that zone was chosen so the fish swimming in Z1 presented a social and non-aggressive behavior. Locomotion and stress were measured by the following parameters: total distance travelled and immobility time. Sociability parameters were assessed by measuring: the time spent and number of entries in the social zone and time spent in Z1 (Figure 2). For both assays, the zebrafish were transported to the behavioral testing room the evening before testing and acclimated for 15 h. Ethovision 14XT (Noldus) was used for tracking and analysis.

1.2.2. Shoaling Test

Group social behavior was analyzed by using the shoaling test [59] in an opaque plastic tank of 61 cm diameter filled with 5 cm of water depth. The zebrafish were transported to the behavioral testing room the evening before testing and acclimated for 15 h. Groups of eight familiar fish (four females, four males) were placed at the same time in the middle of the tank, left to acclimate for 5 min and filmed from above for 10 min with the same setup as the test used for NTT. This experimental setting was modified from Pham and colleagues and Gutiérrez and colleagues [48,60]. The analysis was formed on four different shoals per condition. Ethovision 16XT (Noldus) was used for tracking and measuring locomotion (total distance travelled), stress (immobility time) and inter-individual distances. A custom code for EthoVision XT 16 written in JavaScript, kindly provided by Fabrizio Grieco and Egon Schrasser, was employed for the nearest neighbor distances.

1.3. Acetylcholinesterase Activity

To avoid any neuronal modification that could be caused by anesthetic, the fish were euthanized by hypothermic shock on ice (2–4°C) in agreement with the ethical comity and any opposition from the French Ministry of Research under the reference APAFIS#11488. Their heads were sectioned in the frontal plane and the brain and muscles were dissected under binoculars. The organs ($n = 15–17$ females and males per condition) were homogenized in a homogenization buffer (Phosphate buffer, Glycerol 20% v/v, PMSF 0.2 mM) using a bead crusher (Precellys Evolution, Bertin Technologies, Montigny-le-Bretonneux, France) maintained at 4 °C during homogenization, at 5000 rpm and for two cycles of 20 sec. We used the Bradford method for protein assay to determine the total protein concentration in each sample. The measure of acetylcholinesterase (AChE) activity was performed in 96-well plates as follows. We added 200 µL of Phosphate buffer, 4 µg of protein sample in duplicates or AChE standard and 20 µL of dithiobisnitrobenzoate (0.2 mM final concentration) (DTNB D218200, Sigma, Setagaya City, Japan) before incubation for 10 min at 25 °C. Then, 10 µL of Acetylthiocholine iodide (2 mM final concentration) (ATCi

A5751, Sigma) were added. The hydrolysis of acetylthiocholine was monitored by the formation of the thiolate dianion of DTNB, and the absorbance was read at 412 nm for 3 min at 30 s intervals, as previously described [61]. The AChE activity was expressed as U/mg defined by the number of enzyme units (amount of enzyme that catalyzes the reaction of 1 nmol of substrate per minute) per ml divided by the concentration of protein in mg/mL.

1.4. Library Preparation and RNA Sequencing

The fish were euthanized by hypothermic shock on ice (2–4 °C), their heads sectioned in the frontal plane, and the brain was dissected under binoculars. Each sample consisted of three to four dissected telencephalons formed in four to six replicates, keeping an equilibrated number of males and females per condition. The brains were homogenized in 200 µL of Trizol (Life Technologies, Carlsbad, CA, USA) using beads homogenizer (Precellys Evolution, Bertin Technologies) with two cycles of 30 s each. Total RNA extraction was performed following manufacturer instructions with an additional chloroform extraction before isopropanol precipitation. RNA integrity (RIN), quality and concentration were assessed using RNA Nano Chips (Bioanalyzer 2100, Agilent, Santa Clara, CA, USA). All samples had a RIN > 8. Sequencing libraries were generated from 1 µg of the total RNA following the TruSeq mRNA stranded protocol (Illumina, San Diego, CA, USA). After a quality check and concentration determination on DNA1000 Chips (Bioanalyzer 2100, Agilent, Les Ulis, France), the libraries were run on a HiSeq4000 platform to produce 50 bases long paired-end reads (GenomeEast, IGBMC, Illkirch, France).

1.5. Analysis of Transcriptomics Data

Between 72 and 136 million of good-quality reads ($Q > 30$) were produced for each sample and analyzed as described before [62]. Briefly, the reads quality was assessed with an in-house pipeline to determine quality over read length, duplication rates, insert size, adaptors contamination, nucleotide composition and mapping rates against the reference genome (*RNA-STAR* with GRCz11 and known exon–exon junctions from Ensembl release 98). Normalization and differential expression analysis were performed with *DESeq2* v1.30.1. Biological reproducibility was assessed by the hierarchical clustering of the variance stabilized expression data (rlog) obtained from *DESeq2* with Pearson's correlation and complete linkage method. Genes with $|\text{fold change}| \geq 1.5$ and p -value corrected for multiple testing, $\text{fdr} < 0.05$ (false discovery rate [63]) were considered as differentially expressed in all analysis. Afterwards, functional enrichments was performed using Gene ontology (GO) with R package *clusterProfiler* using zebrafish genes and human orthologues retrieved by Ensembl biomart (as published before, [64–66]) as gene annotation is more abundant in this species. The significance was considered when fdr from Fisher's exact test was < 0.05 . For the volcano-plot of differentially expressed genes (DEG) of neurohormones and neurotransmitters, the following GO terms were used to select genes with the R package *biomaRt* [64] (Supplementary Materials, Table S2). The resulting list of genes was then manually curated to verify gene function.

1.6. RNA In Situ Hybridization on Adult Brain Sections

Antisense RNA probes labelled with digoxigenin (DIG) for *oxt* and *crx* were generated from adult zebrafish brains as follows. Total RNA extraction was performed using TRIzol/chloroform extraction (Life Technologies), with a supplementary chloroform extraction step before performing the isopropyl alcohol precipitation of the nucleic acids. We performed the reverse transcription using SuperScript III (Life Technologies), dNTP, polydT primers and 1 µg of total RNA following manufacturer instructions. PCR amplification was performed using Taq platinum kit (Life Technologies) with 1 µL of RT and the primers pairs: *oxt* forward AATGTCTGGAGGTCTGCTGT, *oxt* reverse TGCCTAATGTACAGTCAAGCT,

crx forward GCTGCTCGGTCTCTTATTGC, *crx* reverse AGTCAAGGCAGTCTACCGAA. PCR products were validated by electrophoresis on 1.2% w/v agarose gel and cloned into a PCRII dual promoter bacterial vector following the kit instructions (TOPO TA Cloning, Invitrogen, Waltham, MA, USA). The transformation of chemically competent TOP10⁺E.coli (Invitrogen) was performed with 2 µL of ligation production. The bacteria were plated on a Petri dish with LB agar with 100 µg/mL Ampicillin and incubated overnight at 37 °C. Five colonies for each construct were picked and grown individually in liquid LB medium with 100 µg/mL of Ampicillin overnight at 37 °C with agitation. MiniPreps (Qiagen DNA MiniPreps, Hilden, Germany) were performed for the extraction of plasmid DNA following the alkaline lysis protocol and sent for Sanger sequencing to check cDNA identity and the orientation of cloning in the PCRII vector. The digestion of the DNA was performed by cutting the DNA plasmid at the 5' end of the cDNA with restriction enzyme: NotI HF, followed by phenol/chloroform extraction of the linearized vector, and in vitro transcription of the Dig RNA probes with Sp6 RNA polymerase and the Dig-UTP labelling mix (Roche, Basel, Switzerland). The technique of in situ hybridization labelling was adapted from ZFin [67]. Two–three brains were used per condition. Briefly, freshly dissected brains were fixed overnight at 4 °C in 4% w/v PAF and transferred in 100% v/v methanol at -20 °C until use. The brains were rehydrated in a decreasing gradient series of methanol diluted in PBS v/v at 70%, 50% and 25% for 5 min each and then washed four times 5 min in PBS with 0.01% v/v Tween 20 (PTW buffer) and incubated with proteinase K at 10 µg/mL in PTW buffer for 30 min and fixed in 4% w/v PAF for 20 min. Five consecutive washes of 5 min in PTW were completed before incubation with hybridization buffer (50% v/v deionized formamide, 5X SSC, 500 µg/mL yeast tRNA, 50 µg/mL heparine, 0.1% v/v Tween20, 9 mM citric acid in water) that lasted until all the brains reached the bottom of the tube (approximately 20 min). The brains were then incubated 4 h at 65 °C in clean RNA hybridization buffer and put in contact overnight with 50 ng (*oxytocin*) or 100 ng (*crx*) of gene specific DIG-labelled RNA probes diluted in RNA hybridization buffer. The brains were washed at 65 °C as follows: twice during 30 min in wash buffer 1 (50% v/v formamide, 1X SSC, 0.05% v/v Tween20), once in wash buffer 2 (2X SSC, 0.1% v/v Tween20) for 15 min, twice in wash buffer 3 (0.2X SSC, 0.1% v/v Tween20) for 30 min and finally once in wash buffer 4 (0.1X SSC, 0.1% Tween20) for 5 min. The brains were then washed 3 times 5 min in PTW at room temperature, incubated 1 h at room temperature in blocking buffer (1X PBS, 0.1% v/v Tween20, 0.2% v/v BSA, 1% v/v DMSO), embedded into 2% agarose and sectioned at 70 µm with a vibratome (VT1200S Leica Biosystems, Wetzlar, Germany) in a coronal plane. The brain sections were incubated 1h in blocking buffer, then overnight at 4 °C with anti-Digoxigenin-AP Fab fragment antibody (Roche Diagnostics GmbH, Mannheim, Germany) diluted at 1:4000 in blocking buffer and washed 5 times 15 min with PTW buffer before staining with NBT/BCIP (Roche Diagnostics GmbH, Germany). The slides were mounted with Vectashield HardSet (Vector Laboratories, Newark, CA, USA, ref: H-1400). Each in situ hybridization staining was repeated at least two times. The expression patterns were observed under a Leica binocular (DM750) equipped with a Leica ICC50 camera at x10, x20 and x40 zoom. For the quantification of the intensity signal of oxytocin, we performed a fluorescent RNA in situ hybridization. The protocol is similar as the one previously described except that before adding the hybridization buffer, the samples were incubated for one hour with 3% hydrogen peroxide (H₂O₂, Sigma) in PTW and then washed three times 10 min in PTW; anti-digoxigenin-AP was substituted by Anti-digoxigenin-POD (poly) Fab fragments (Roche Diagnostics GmbH, Germany) diluted at 1:1000 in blocking buffer and before staining with TSA Plus Cyanine 3 (Perkin Elmer, Boston, MA, USA),

samples were rinsed twice 10 min in 0.002% H₂O₂ in PTW. Zeiss Confocal LSM780 was used with laser 561 at x40 zoom. We first checked for autofluorescence and background noise without detecting either of them.

1.7. Statistical Analysis

For the behavioral analysis, and especially novel tank and social preference tests, a first statistical analysis by a two-way ANOVA test indicated significant sex differences (data not shown). This is in agreement with other studies that already showed distinct behavioral profiles between males and females [68,69]. We thus formed analyses for each sex separately. Linear effects models with Gaussian family were used for normally distributed data (total distance travelled in NTT, latency to first entry to the top zone, time in Z1, time of immobility) or with sqrt transformation to reach normality when needed (time in top zone, total distance travelled for the social preference test). For over-dispersed counted data, the generalized linear mixed effects model for the negative binomial family was used (number of entries in top or social zone). As the experiments were performed in two different batches, we added a random effect corresponding to the session date. p -values < 0.05 were considered significant.

Regarding the Shoaling test, statistical analysis was formed by applying a generalized linear mixed model with Gamma distribution for continuous and positive data. Since individuals show more behavioral similarities among one shoal, the shoals were used as a random effect in the model. p -values < 0.05 were considered significant.

The data corresponding to acetylcholinesterase activity analysis being normally distributed (except for female muscles that needed log transformation), the analysis was performed on each sex separately by one-way ANOVA followed by a Dunnett post-hoc test. p values < 0.05 were considered significant.

For the quantification of fluorescent RNA in situ hybridization of *oxytocin*, equal regions of interest (ROIs) were selected on several vibratome slices of three brains per condition and the integrated density for each ROI was determined by dividing the intensity of ROI to a representative area without signal (the removal of background noise). Statistical analysis was undertaken by applying a generalized linear mixed model with Gamma distribution for continuous and positive data. Since several slides originated from the same brain, brains were used as a random effect in the model. p -values < 0.05 were considered significant.

3. Results

1.8. Chronic Irradiation Altered mRNA Expression in Adult Zebrafish Telencephalon

The modulation of transcriptional expression in adult telencephalons was assessed by RNAseq after continuous exposure to gamma radiation for 36 days at 0.05 mGy/h (D005), 0.5 mGy/h (D05) and 5 mGy/h (D5). A dose rate-dependent increase in differentially expressed genes (DEG) was observed with 27 DEG at 0.05 mGy/h, 200 DEG at 0.5 mGy/h and 530 DEG at 5 mGy/h ($|\text{fold change}| \geq 1.5$ and $\text{fdr} < 0.05$). A relatively high number of DEG was common between 0.5 mGy/h and 5 mGy/h ($n = 156$ genes, 26%) while very few DEG were shared with the 0.05 mGy/h condition (Figure 1a).

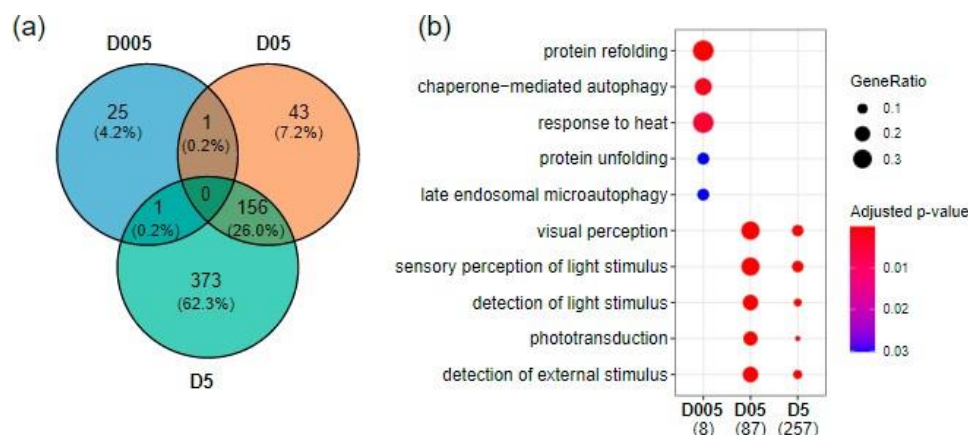


Figure 1. Transcriptomics effects of radiation exposure on zebrafish telencephalon. (a) Venn diagram of differentially expressed genes at the three dose rates ($|\text{fold change}| \geq 1.5$ and adjusted p -value < 0.05). (b) GO enrichment of biological processes using human orthologues showing the top enriched pathways at D005 (0.05 mGy/h), D05 (0.5 mGy/h) and D5 (5 mGy/h). The numbers in brackets correspond to the number of genes present in the enriched GO terms. The dot size corresponds to the fraction of genes in a given GO term compared to all DEG (GeneRatio).

The enrichment of Gene Ontology (GO) Molecular Function and Biological Process, either with *Danio rerio* genes or via human orthologous genes, demonstrated that the pathways altered after chronic exposure to IR at D05 and D5 included visual perception (GO:0007601; D05: $\text{fdr} < 10^{-22}$ D5: $\text{fdr} < 10^{-17}$), the regulation of G protein-coupled receptor signaling pathways (GO:0008277; D05: $\text{fdr} < 10^{-8}$; D5: $\text{fdr} < 10^{-8}$) and serotonin metabolic process (GO:0042428; D05: $\text{fdr} = 0.002$; D5: $\text{fdr} = 0.03$) (Figure 1b and Supplementary Table S3). We also detected other biological processes that were significantly enriched such as retinoid binding (GO:0005501; D05: $\text{fdr} = 10^{-5}$; D5: $\text{fdr} = 10^{-4}$), the regulation of axon extension involved in axon guidance (GO:0048841, D05: $\text{fdr} = 0.01$; D5: $\text{fdr} = 0.03$) and optokinetic behavior (GO:0007634; D05: $\text{fdr} = 0.01$; D5: $\text{fdr} = 0.03$) (Supplementary Table S3). In contrast, pathways enriched after irradiation at 0.05 mGy/h highlighted the dysregulation of genes involved in protein refolding (GO:0042026; $\text{fdr} < 10^{-4}$).

1.1. Chronic Irradiation Induced Hypolocomotion

Since transcriptomics data revealed changes in gene expression in the brain after irradiation, we assessed if these modifications could result in potential changes in behavior. Since DEGs were mostly observed when exposing at 0.5 mGy/h and 5 mGy/h, we focused on these two dose rates as modifications after 0.05 mGy/h radiation perturbed the expression of few genes ($n = 27$). First, locomotion was measured as an integrated parameter to evaluate the effects of radiation on adult brain function and behavior. The total distance travelled was measured by three different behavioral tests on adult zebrafish, the shoaling test (ShT), the Novel Tank test (NTT) and the social preference test (SP) (Figure 2).

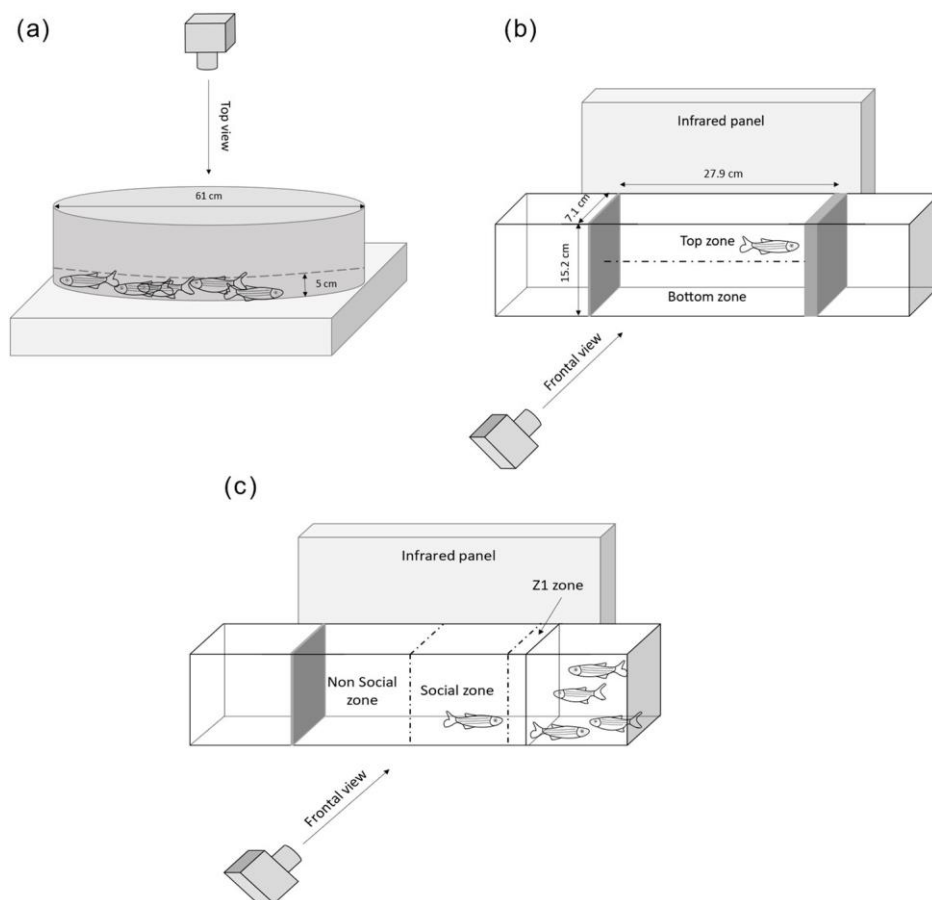


Figure 2. Experimental setup of the three behavioral tests. (a) The shoaling test, (b) the novel tank test, (c) the social preference test [57–59].

The total distance travelled was decreased at both dose rates in the ShT (D05: $t = -13$, $p = 10^{-16}$; D5: $t = -4.7$, $p = 10^{-6}$, Figure 3a). Similar trends were also detected in the females exposed in NTT at 0.5 mGy/h ($t = -2.7$, $p = 0.01$) and 5 mGy/h ($t = -1.4$, $p = 0.17$) and in the SP (D05: $t = -1.5$, $p = 0.13$; D5: $t = -2.7$, $p = 0.01$), but not in males (Figure 3b–e). Since acetylcholine is involved in muscle contraction and in brain function by participating in anxiety-like behavior or social response [70,71], the activity of acetylcholinesterase (AChE) was measured in both the muscle and brain after irradiation. Decreased reduction in enzymatic activity can be observed in the brain and muscle of exposed females at both dose rates but this decrease is not statistically significant (in brain: $F = 2.5$, $p = 0.11$; in muscles: $F = 2.77$, $p = 0.09$, Figure 3f,g). We did not observe any effect in the brain or muscles in males (brain: $F = 0.15$, $p = 0.86$; muscles: $F = 0.78$, $p = 0.47$, Figure 3h,i).

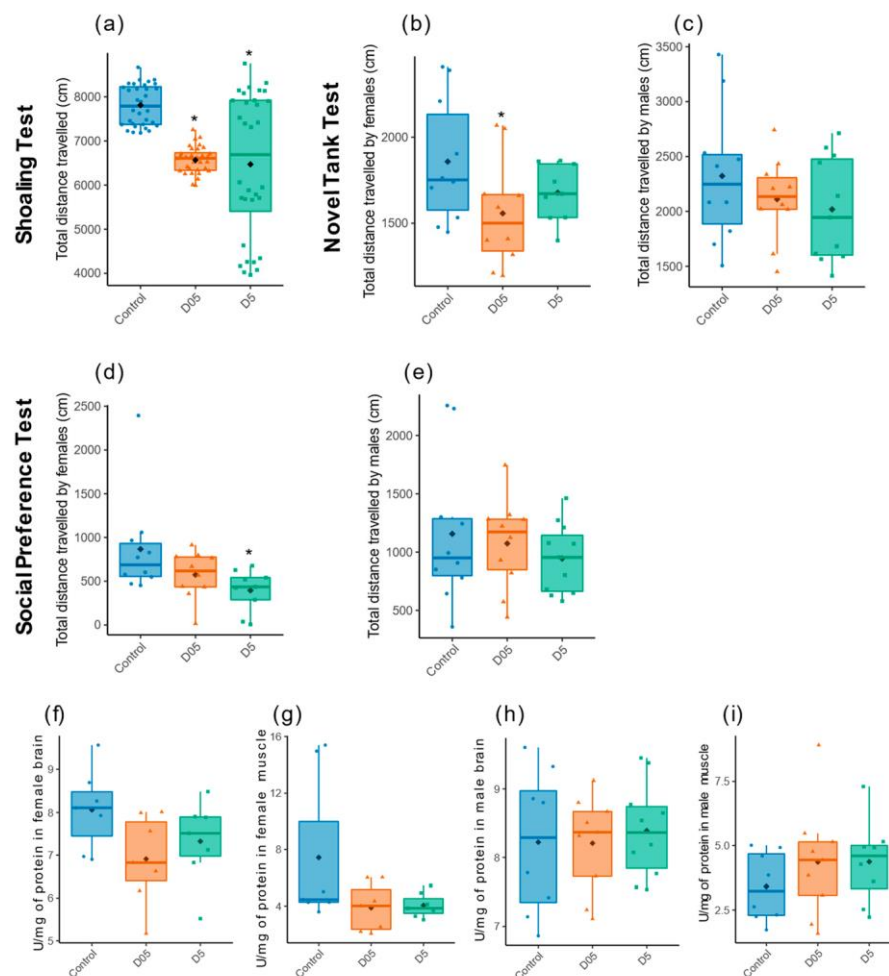


Figure 3. Effect of radiation exposure on locomotor activity and dosage of Acetylcholinesterase activity. Total distance travelled in centimeter (cm) was evaluated by (a) Shoaling test ($n = 32$), (b,c) Novel tank test and (d,e) Social preference test in females zebrafish ($n = 9$ – 10) and males ($n = 10$ – 11). Data are represented by mean (black point), median (horizontal line) and ± 75 th and 25th percentiles, and significance assessed by Gamma generalized linear mixed models (a) or Gaussian linear effects models (b–e). Measure of AChE activity in (f) brain and (g) muscle of females, and in males (h,i). Data are represented by mean (black point), median (horizontal line) and ± 75 th and 25th percentiles of 7 individuals per condition. AChE activity is shown as enzymatic activity Unit per mg of protein (U/mg protein). Statistical analysis was performed by using one-way ANOVA followed by Dunnet post-hoc test. * $p < 0.05$.

1.9. Chronic Irradiation Increases Stress Behavior in Female Zebrafish

We then evaluated the stress potentially induced by IR by measuring the number of entries, the latency to first entry and the time spent in top zone in the NTT. The number of entries in the top zone reached statistical significance only for females exposed at 0.5 mGy/h (Figure 4, $t = -3.1$, $p = 0.002$).

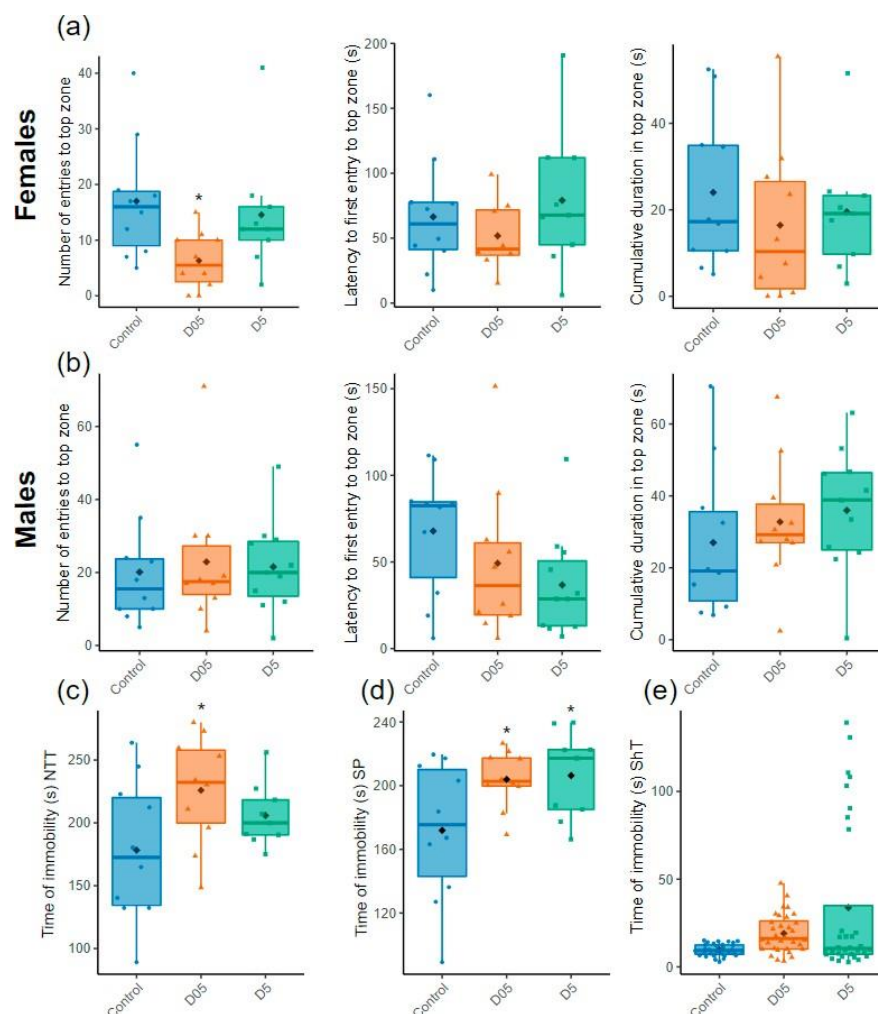


Figure 4. Assessment of stress behavior. **(a)** Number of entries, latency to first entry and time spent in the top zone in the novel tank test (NTT) for females and **(b)** males ($n = 9–11$). **(c)** Time of immobility of females in the NTT ($n = 9–10$), **(d)** the Social Preference test (SP) ($n = 9–10$ females or males) and **(e)** the Shoaling test (ShT) ($n = 32$ both females and males). Data are represented by mean (black point), median (horizontal line), ± 75 th and 25th percentiles and analyzed with negative binomial mixed effect models (**a,b**, left panels) or Gaussian linear effects models (**a,b**, middle and right panel, **c,d**) or Gamma generalized linear mixed models (**e**). * $p < 0.05$.

The other parameters measured in the NTT (latency to first entry and cumulative duration in the top zone) were not altered (Figure 4a,b), middle and right panel). Immobility is often used to evaluate anxiety-like behavior in zebrafish [72]. We thus assessed the relative time of immobility (freezing) in the three behavioral tests as another way to evaluate stress after chronic exposure to radiation. An increase in immobility was observed in females but not in males at both dose rates in the SP (D05: $t = 2.3$, $p = 0.03$; D5: $t = 2.4$, $p = 0.03$, Figure 4d). A similar increase was also observed in the NTT, especially at 0.5 mGy/h (D05: $t = 2.7$, $p = 0.01$; D5: $t = 1.4$, $p = 0.17$, Figure 4c), as well as in the ShT but this alteration was not statistically significant (D05: $t = -1.4$, $p = 0.17$; D5: $t = -0.54$, $p = 0.59$, Figure 4e).

1.10. Chronic Irradiation Reduces Sociability in Female Zebrafish

The zebrafish being a social animal with complex behavior, the proximity of fish with conspecifics was measured to assess sociability in the SP test. We observed that the time spent close to the conspecifics (Z1) was decreased for females in both irradiated conditions but only significant in 0.5 mGy/h of the irradiated fish (D05: $t = -2.7$, $p = 0.01$ and D5: $t = -1.9$, $p = 0.06$, Figure 5a). We also measured the time spent and number of entries with a one-body-length zone close to the conspecifics and observed a significant decrease in entries in 5 mGy/h of the irradiated fish (data not shown).

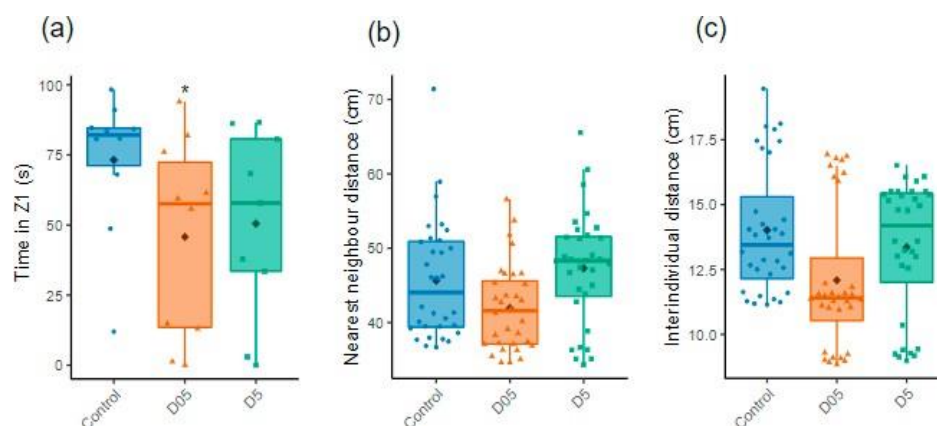


Figure 5. Assessment of individual and group social behavior. (a) Time in Z1 evaluated for females during the Social Preference test ($n = 9$ – 10). (b) Nearest neighbor distances and (c) Inter-individual distances analyzed during the Shoaling test (ShT) ($n = 32$). Data are represented by mean (black point), median (horizontal line), ± 75 th and 25th percentiles and analyzed with Gaussian linear effect model (a), Gamma generalized linear mixed models (b,c). * $p < 0.05$.

A significant decrease in the number of entry in the social zone was also detected using a broader zone at 5 mGy/h ($t = -2.4$, $p = 0.02$, data not shown). We did not observe any modifications in the males. We then used the ShT to assess group cohesion within a shoal. No alterations in the social group was observed using either nearest neighbor or inter-individual distances (Figure 5b,c).

1.11. Induction of Gene Expression Involved in Stress Response or Neurogenesis

Since the zebrafish displayed stress behavior and the transcriptomic profile of the brain radiation response suggested the dysregulation of genes involved in neurotransmission (including serotonin), we explored the changes of neurohormones and neurotransmitters expressions in the RNAseq dataset (Supplementary Materials, Table S2. The significant modulation of eight genes coding for proteins with a function in neurohormone synthesis, uptake, metabolism and receptor production, or in neurotransmission was found at 0.5 mGy/h and thirty-two genes at 5 mGy/h (Figure 6a,b and Supplementary Table S4).

These included the genes coding for the enzymes *tryptophan hydroxylase 1a* (*tph1a*) and *tryptophan hydroxylase 2* (*tph2*) that control brain serotonin synthesis, as well as the neurohormones oxytocin (*oxt*) and arginine vasopressin (*avp*) (Figure 6c). We also detected a variation in the expression of *cyp11c1* involved in cortisol and 11-ketotestosterone (11-KT) production, *asip2b* known to regulate cortisol secretion and the stress-induced transcription factor *nr4a1* [73–76]. To further explore how radiation can impact gene expression in the brain, and validate the RNAseq data, we performed RNA in situ hybridization on adult brain sections for *oxt*, a gene expressed in the preoptic area and involved in both the regulation of stress and social behavior [77]. At 5 mGy/h, we observed an ectopic expression of *oxt* in the ventricular zone (VZ) of the anterior part of the

parvocellular preoptic nucleus (PPa)(Figure 7a,a') where neural progenitors are located [78]. Indeed, in the control condition, cells lining the VZ do not express oxt, while after irradiation, oxt expression is detected. To quantitatively confirm this observation, we measured oxt expression in control and irradiation (5 mGy/h) by fluorescent RNA in situ hybridization and detected a significant increase in signal intensity (Supplementary Materials, Figure S1).

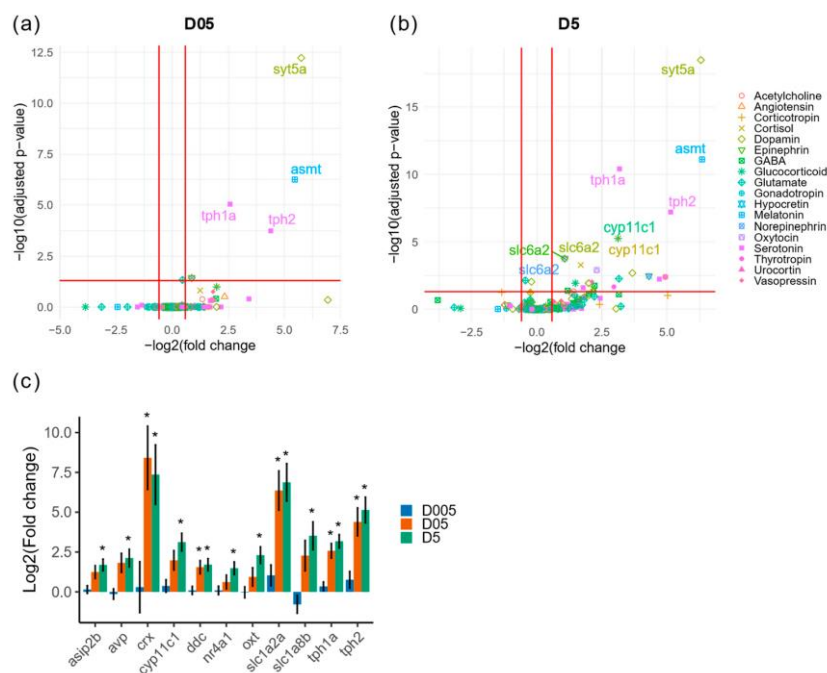


Figure 6. Transcriptomic analysis of genes with function in neurotransmission or neurohormones. (a) Volcano plot of differentially expressed genes with function in neurotransmission or brain neurohormones at D05 (0.5 mGy/h) and (b) at D5 (5 mGy/h). The biological process associated to each gene is indicated by the color and shape code. (c) Change in expression of dysregulated genes. * Indicate adjusted p -value (<0.05).

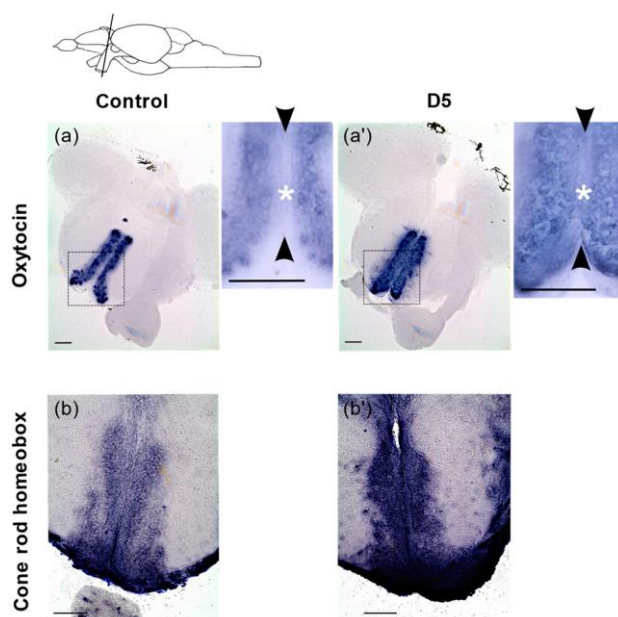


Figure 7. RNA in situ hybridization on adult brain sections showing expression of *oxt* and *crx* expressing cells. (a,a') Coronal sections of brain showing expression of *oxt* in the anterior parvocellular preoptic nucleus (PPa) in control and 5mGy/(D5) irradiated fish. The black rectangle represents the area for the higher magnification images. (b,b') Expression of *crx* in the PPa in the control and after irradiation at 5 mGy/h(D5). The black arrow heads delineate the ventricular zone (VZ) in the medial ventricular zone of the PPa and the white asterisk represents the location of VZ progenitors where ectopic expression is detected. Scale bar = 100 μ m. The scheme at the top represents the location of the coronal sections in the brain.

Similarly, the gene *cone-rod homeobox (crx)*, known to be involved in neuronal differentiation in the retina [76], was strongly upregulated in the PPa in both the VZ as well as in cells located laterally to the VZ (Figure 7b,b'). These results confirm the upregulation of *oxt* and *crx* observed at 5mGy/h by the transcriptomic analysis.

4. Discussion

In this study, we used adult zebrafish to better characterize the effects of chronic low to moderate dose rates of IR on brain function and behavior. We employed a multiscale approach that characterized the effects on behavior, including locomotion, the assessment of the stress response and sociability, completed by a transcriptomic analysis on the telencephalon to characterize the effects at the molecular scale. This analysis was completed by a detailed mapping of the gene expression of two candidate genes using RNA in situ hybridization on adult brain sections. This strategy allowed us to assess which molecular pathways were dysregulated following chronic exposure to low and moderate dose rates of IR and helped in deciphering the molecular basis of changes in adult behavior.

Transcriptomic analysis highlighted the dysregulation of genes with function in synaptic plasticity, including axon guidance signaling (*arl3l2*, *arl3l*, *sema3d* or *unc119.2*) or the regulation of the G protein-coupled receptor signaling pathway (*cng1*, *pde6b*, *grk7*, *guca1c*). It is well known that synaptic plasticity is involved in organisms' adaptation and, when disturbed, may lead to pathological behaviors such as stress-related disorders [79,80]. A previous study in mice exposed acutely to 100 mGy detected similar perturbation, but in contrast to our results, gene expression was mostly downregulated in the rodents [30]. Additionally, high doses of acute exposure includes the induction of neuro-inflammation with the overexpression of pro-inflammatory genes [81,82], cellular senescence [83], the decrease in adult neurogenesis possibly due to the apoptosis of proliferating cells [82,84], DNA damage like double strand breaks [10,85] and oxidative stress through the elevation of pro-oxidants or lipid peroxidation [86,87]. Interestingly, in our study, we could not detect significant change in these processes through the transcriptomic analysis. Possibly, the different exposure scenarios (chronic versus acute) or the use of young 8- to 10- week mice (while we used sexually mature organisms) explain this difference, but more likely, given the very high regenerative capacity of the adult zebrafish brain in comparison to juvenile rodent [88–90], the genes involved in the axon guidance and G protein-coupled receptor signaling were only upregulated in fish, which might reflect a specific response to the insults caused by IR. This hypothesis of induced regenerative process after irradiation is supported by the very strong upregulation of *crx* in the adult telencephalon. Indeed, this gene is a well-known transcription factor (TF) of the *otx*-like homeodomain family that regulates the neuronal differentiation of photoreceptors in the retina [91]. However, its role in the adult zebrafish telencephalon is currently unknown. Nevertheless, its expression in cells lining the ventricular zone (VZ) as well as in cells located more laterally in the PPa corresponds to the expression domain of neuronal progenitors (lining the VZ) and differentiating neurons (laterally to the VZ). The fact that *crx* is strongly upregulated in the PPa after irradiation could thus indicate the production of new neurons in this region of

the adult brain and highlights its potential role in neurogenesis. If these transcriptomic modifications lead to changes at the protein level remains to be investigated. More analysis, including double labelling with progenitors and differentiation markers, is needed in order to confirm or infirm this regenerative hypothesis. We observed a hypolocomotion state noticeable by a reduced total distance travelled at dose rates equal to and higher than 0.5 mGy/h in the ShT, which was also detectable in the SP and the NTT. Interestingly, similar effects on locomotion were identified in 5-day-old larvae exposed to continuous radiation at the same dose rates [62]. Moreover, hypolocomotion was observed in mice 6 h after acute exposure to 500 mGy or 2000 mGy [92], and up to 18 months after irradiation of 10-week-old mice at 500 mGy [93]. Thus, our data complement the existing knowledge of IR by showing the effects on locomotion in adults in the field of low dose rates. Our analysis on AChE activity, the enzyme involved in the degradation and recycling of acetylcholine that acts both in the brain and neuromuscular junctions, demonstrated a marginal decrease in enzymatic activity in the female muscles and brain at 0.5 mGy/h and 5 mGy/h. In males, a non-significant increase in AChE activity was observed in the muscles at 5 mGy/h, and we did not see any effect on locomotion or stress. Together, our data highlight chronic irradiation-induced hypolocomotion in females, and this effect was substantially independent of possible alterations at the neuromuscular junction. This result contrasts with previous data that showed decreased AChE activity in zebrafish larvae exposed at 0.01 mGy/h [94].

The results on stress behavior from the NTT revealed a decreased number of entries in the top zone after the exposure of females at 0.5 mGy/h, suggestive of increased stress, but no change could be observed at 5 mGy/h, and males were not affected. If a non-linear dose rate effect could be proposed for females, it remains possible that insufficient individual data impaired the analysis. The cumulative duration of immobility (also called freezing) is another parameter used to measure stress [95]. Our data demonstrated an increased cumulative time of freezing in females irradiated at 0.5 mGy/h in the NTT, but, as before for locomotion, no effect could be detected in males or at 5 mGy/h. However, by measuring this endpoint in the SP, we could detect a significant increase in freezing both at 0.5 mGy/h and 5 mGy/h in females. An increased cumulative duration of immobility was also apparent in the ShT, but these results were not statistically significant, presumably because males' and females' behavior cannot be distinguished in this group behavior test. The hypolocomotion is thus presumably a consequence of increased freezing time. These results show that chronic irradiation at 0.5 mGy/h, and presumably higher dose rates, induces stress-like behavior in female zebrafish but not in males. At the molecular level, stress behavior is linked to increased cortisol release. Under stress, an increase in CRH levels results in higher glucocorticoid hormone (GC) secretion (cortisol or corticosterone depending on species) which bind in the plasma to corticosteroid-binding globulin (CBG) and albumin [96]. As teleost have a developed hypothalamus-pituitary-interrenal axis and use cortisol as their primary stress hormone [73,97–101], the increasing freezing time observed in our data is very likely to be linked to an elevation of cortisol secretion. The fact that several genes involved in glucocorticoid production were upregulated in the brain of the irradiated zebrafish, including *cyp11c1*, *nr4a1*, *asip2b*, *avp* and *oxt*, is also in favor of such interpretation and furthermore can provide a mechanistic explanation for the increased stress behavior observed at dose rates equal to and higher than 0.5 mGy/h. These results are in agreement with previous data on the chronic exposure of meadow voles at 22.6 μ Gy/h [102], which demonstrated higher levels of free corticosterone in females than in males, along with elevated corticosteroid-binding globulin (CBG) (in females). The results in meadow voles are thus suggestive of a chronic stress response visible by the prolonged production of GC and its transport in the plasma. The evaluation of such a response in the context of chronic irradiation remains to be determined.

The use of RNA in situ hybridization on adult brain demonstrated an upregulation of *oxt* in the anterior part of the parvocellular preoptic nucleus (PPa). It expresses several neurohormones such as oxytocin or arginine-vasopressin with conserved function compared

to mammals [103] such as the regulation of water homeostasis [104], the control of blood pressure [105] as well as a role in social behavior and the regulation of anxiety [49,50,106]. Interestingly, a recent analysis of the stress response in zebrafish correlated to the increased number of oxytocinergic cells in the brain with environmental stress provoked by immersion in deionized water for 24 h [50]. Other experiments in rodents revealed that repeated defeat experiences increased the number of oxytocinergic neurons in the PVN as well as after chronic homotypic stress [107,108]. From these studies, it was proposed that acclimation to chronic stress might involve the upregulation of oxytocin expression in the hypothalamus. Therefore, chronic low to moderate dose rates of gamma radiation can induce the upregulation of *oxtr* observed in the VZ of the PPa which could thus reflect the acclimation of fish to chronic stress.

The SP test revealed a decreased sociability of adult fish at both dose rates. No change in group behavior could be detected in the ShT, presumably because no distinction could be established between males and females in this test. Mechanistically, it is tempting to speculate that this change in social behavior is linked to changes in the expression of *oxytocin* we observed in the cells of PPa. Indeed, oxytocin has a dual role in anxiety-like behavior and in social behavior in both mammals [109–111] and zebrafish [49,51,112]. For instance, the knockout of one of the oxytocin receptors (*oxtr* *-/-* or *oxtr1* *-/-*) enhanced the social preference level in zebrafish larvae at 3 and 4 weeks post fertilization [51]. Similarly, the early life ablation of oxytocin neurons led to a reduced social preference in adult zebrafish [113]. However, contrasting results in social behavior are obtained depending on the experimental design [49,51,114], which means it is more difficult to predict if an increase in oxytocin production will lead to increase or decrease in social preference. Beside oxytocin, we also noted the upregulation of *avp* expression in the brain after irradiation at 5 mGy/h (by RNAseq). This neuropeptide can stimulate ACTH secretion from the fish pituitary, and it was shown that after acute stress, such as confinement or enforced displacement, the *avt* transcript is induced in parvocellular AVT neurons in the rainbow trout [115,116]. Moreover, arginine vasopressin was also proposed to regulate social behavior since injecting *avp* in zebrafish induced social interaction [48]. Several studies suggested crosstalk between *avp* and *oxtr* signaling in the modulation of social behavior [49,117,118], suggesting that both systems might act in synergy in the increased anxiety observed after irradiation. In zebrafish, oxytocin has been proven to not take part in the regulation of the stress response when acting through its receptors [49,119]. However, we cannot rule out that a decrease in the degree of sociability could be an indirect consequence of the stress response observed. Functional studies could be useful to precisely understand if these two responses (stress and sociability) are independent in the context of chronic low and moderate dose rates of ionizing radiation.

Finally, transcriptomic analysis also revealed the upregulation of *tph1a* and *tph2* at 0.5 mGy/h and 5 mGy/h, two genes coding for enzymes essential for serotonin synthesis. The serotonin levels in the brain were correlated to the maturation of shoaling behavior during zebrafish development [120]. Moreover, the interactions between the serotonin and oxytocin systems were suggested in humans and mice [46,47]. Serotonin plays also a role in the stress response by the activation of the HPA axis in mammals and studies in zebrafish with 5HT agonist/antagonist receptors showing an impact on anxiety-like behaviors [121,122]. Our results also showed the dysregulation of the transporter *slc18A2*, also known as *vesicular monoamine transporter 2* (or *VMAT2*), which transports either serotonin or dopamine. The knockout of the dopamine transporter (DAT) in zebrafish resulted in a chronic anxiety-like state [123]. Hence, the modification of serotonin and dopamine availability might participate in the behavioral patterns observed. The stress response observed after irradiation involved thus a complex interaction between various neurohor-

mones and neurotransmission, which together drive the acclimation of fish subjected to chronic environmental stress.

5. Conclusions

In this study, we aimed at elucidating the relation between the molecular and behavioral effects under chronic low to moderate dose rates of IR. Our results show the emergence of a stress response, highlighted by genes known to be involved in the HPI axis or coding for neurohormones (oxytocin, arginine-vasopressin) and neurotransmitters (serotonin, dopamine but not acetylcholine) that play a role in the regulation of anxiety-like behavior. This stress response is translated at the individual scale by hypolocomotion, increased freezing and social stress. We demonstrated that the effects on locomotion were not linked to the alteration of AChE activity in the brain or at the level of neuromuscular junctions. Finally, we confirmed by RNA in situ hybridization the increased expression of *oxt* and *crx* in the VZ of the PPa, suggestive of modulations of neurohormone production in the PPa to regulate stress behavior. Together, these findings highlight the intricate interaction between neurohormones, neurotransmission and neurogenesis to balance disturbed brain homeostasis caused by chronic exposure to IR.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/cancers14153793/s1>, Table S1: Effective dose rates estimated by MCNP; Table S2: Go terms associated to neurohormones or neurotransmitters used for the production of the volcano-plots of differentially expressed genes; Table S3: Enrichment of GO pathways (Biological process and Molecular Function) using zebrafish genes and human orthologues; Table S4: Differential expression analysis of neurohormones and neurotransmitters. The process (from Gen Ontology) is indicated for each gene; Figure S1: Quantification of signal intensity of *oxytocin* by determining the integrated density.

Author Contributions: Conceptualization, E.C. and O.A.; methodology, E.C., O.A., N.D. and X.C. (behavioral tests); formal analysis, E.C. and O.A.; investigation, E.C., O.A., I.C., V.C. and B.G.; data curation, E.C. and O.A.; writing—original draft preparation, E.C.; writing—review and editing, O.A.; visualization, E.C.; supervision, O.A., C.A.-G. and T.D.C.; project administration, O.A. and C.A.-G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was performed in the framework of the IRSN (Institut de Radioprotection et de Sûreté Nucléaire) working program.

Institutional Review Board Statement: All experiments were approved by the ethical comity (C2EA, IRSN, France) and received agreement from the French Ministry of Research under the reference APAFIS#11488.

Data Availability Statement: Transcriptomics data are available under the Gene Expression Omnibus GEO repository: GSE206573.

Acknowledgments: The authors would like to thank Olivier Simon and Noémie Guirandy and other members of the LECO for their help in the welfare of animals; all the persons involved in the MICADO facility at IRSN; Fabrizio Grieco and Egon Schrasser (Noldus) for a custom code for EthoVision XT 16; Laurent Garcia Sanchez of the LR2T (IRSN) for checking statistical analysis; Magali Coquillat (IRSN) for zebrafish brain drawing; Christian Godon and the support for the microscopy equipment that was provided by the Région Provence Alpes Côte d’Azur, the Conseil General of Bouches du Rhône, the French Ministry of Research, the CNRS and the Commissariat à l’Energie Atomique et aux Energies Alternatives.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Folley, J.H.; Borges, W.; Yamawaki, T. Incidence of Leukemia in Survivors of the Atomic Bomb in Hiroshima and Nagasaki, Japan. *Am. J. Med.* **1952**, *13*, 311–321. [[CrossRef](#)]
2. Ozasa, K.; Shimizu, Y.; Suyama, A.; Kasagi, F.; Soda, M.; Grant, E.J.; Sakata, R.; Sugiyama, H.; Kodama, K. Studies of the Mortality of Atomic Bomb Survivors, Report 14, 1950–2003: An Overview of Cancer and Noncancer Diseases. *Radiat. Res.* **2012**, *177*,

- 229–243. [\[CrossRef\]](#)
3. Grant, E.J.; Brenner, A.; Sugiyama, H.; Sakata, R.; Sadakane, A.; Utada, M.; Cahoon, E.K.; Milder, C.M.; Soda, M.; Cullings, H.M. Solid Cancer Incidence among the Life Span Study of Atomic Bomb Survivors: 1958–2009. *Radiat. Res.* **2017**, *187*, 513–537. [\[CrossRef\]](#)
 4. Otake, M.; Schull, W.J. Radiation-Related Brain Damage and Growth Retardation among the Prenatally Exposed Atomic Bomb Survivors. *Int. J. Radiat. Biol.* **1998**, *74*, 159–171. [\[CrossRef\]](#)
 5. Møller, A.P.; Bonisoli-Alquati, A.; Rudolfson, G.; Mousseau, T.A. Chernobyl Birds Have Smaller Brains. *PLoS ONE* **2011**, *6*, e16862. [\[CrossRef\]](#)
 6. Hayama, S.; Tsuchiya, M.; Ochiai, K.; Nakiri, S.; Nakanishi, S.; Ishii, N.; Kato, T.; Tanaka, A.; Konno, F.; Kawamoto, Y.; et al. Small Head Size and Delayed Body Weight Growth in Wild Japanese Monkey Fetuses after the Fukushima Daiichi Nuclear Disaster. *Sci. Rep.* **2017**, *7*, 3528. [\[CrossRef\]](#) [\[PubMed\]](#)
 7. UNSCEAR. *Report of the United Nations Scientific Committee on the Effects of Atomic Radiation 2010: 57th Session: Summary of Low_Dose Radiation Effects on Health*; UNSCEAR: New York, NY, USA, 2010.
 8. Beresford, N.A.; Copplestone, D. Effects of Ionizing Radiation on Wildlife: What Knowledge Have We Gained between the Chernobyl and Fukushima Accidents? *Integr. Environ. Assess. Manag.* **2011**, *7*, 371–373. [\[CrossRef\]](#)
 9. Cannon, G.; Kiang, J.G. A Review of the Impact on the Ecosystem after Ionizing Irradiation: Wildlife Population. *Int. J. Radiat. Biol.* **2022**, *98*, 1054–1062. [\[CrossRef\]](#)
 10. Verreet, T.; Verslegers, M.; Quintens, R.; Baatout, S.; Benotmane, M.A. Current Evidence for Developmental, Structural, and Functional Brain Defects Following Prenatal Radiation Exposure. *Neural Plast.* **2016**, *2016*, 1243527. [\[CrossRef\]](#)
 11. Passemard, S.; Perez, F.; Gressens, P.; El Ghouzzi, V. Endoplasmic Reticulum and Golgi Stress in Microcephaly. *Cell Stress* **2019**, *3*, 369. [\[CrossRef\]](#)
 12. Hossain, M.; Chetana, M.; Devi, P.U. Late Effect of Prenatal Irradiation on the Hippocampal Histology and Brain Weight in Adult Mice. *Int. J. Dev. Neurosci.* **2005**, *23*, 307–313. [\[CrossRef\]](#)
 13. Schmitz, C.; Born, M.; Dolezel, P.; Rutten, B.; de Saint-Georges, L.; Hof, P.; Korz, H. Prenatal Prolonged Irradiation at Very Low Dose Rate induces Severe Neuronal Loss in Rat Hippocampus and Cerebellum. *Neuroscience* **2005**, *130*, 935–948. [\[CrossRef\]](#)
 14. Brizzee, K. Quantitative Histological Studies on Delayed Effects of Prenatal X-Irradiation in Rat Cerebral Cortex. *J. Neuropathol. Exp. Neurol.* **1967**, *26*, 584–600. [\[CrossRef\]](#) [\[PubMed\]](#)
 15. Lazarini, F.; Mouthon, M.-A.; Gheusi, G.; de Chaumont, F.; Olivo-Marin, J.-C.; Lamarque, S.; Abrous, D.N.; Boussin, F.D.; Lledo, P.-M. Cellular and Behavioral Effects of Cranial Irradiation of the Subventricular Zone in Adult Mice. *PLoS ONE* **2009**, *4*, e7017. [\[CrossRef\]](#) [\[PubMed\]](#)
 16. Baskar, R.; Devi, P.U. Influence of Gestational Age to Low-Level Gamma Irradiation on Postnatal Behavior in Mice. *Neurotoxicol. Teratol.* **2000**, *22*, 593–602. [\[CrossRef\]](#)
 17. Mele, P.C.; Franz, C.G.; Harrison, J.R. Effects of Ionizing Radiation on Fixed-Ratio Escape Performance in Rats. *Neurotoxicol. Teratol.* **1990**, *12*, 367–373. [\[CrossRef\]](#)
 18. Hall, P.; Adami, H.-O.; Trichopoulos, D.; Pedersen, N.L.; Lagiou, P.; Ekbohm, A.; Ingvar, M.; Lundell, M.; Granath, F. Effect of Low Doses of Ionising Radiation in Infancy on Cognitive Function in Adulthood: Swedish Population Based Cohort Study. *BMJ* **2004**, *328*, 19. [\[CrossRef\]](#)
 19. Almond, D.; Edlund, L.; Palme, M. Chernobyl's Subclinical Legacy: Prenatal Exposure to Radioactive Fallout and School Outcomes in Sweden. *Q. J. Econ.* **2009**, *124*, 1729–1772. [\[CrossRef\]](#)
 20. Heiervang, K.S.; Mednick, S.; Sundet, K.; Rund, B.R. Effect of Low Dose Ionizing Radiation Exposure in Utero on Cognitive Function in Adolescence. *Scand. J. Psychol.* **2010**, *51*, 210–215. [\[CrossRef\]](#)
 21. Gamache, G.L.; Levinson, D.M.; Reeves, D.L.; Bidyuk, P.I.; Brantley, K.K. Longitudinal Neurocognitive Assessments of Ukrainians Exposed to Ionizing Radiation after the Chernobyl Nuclear Accident. *Arch. Clin. Neuropsychol.* **2005**, *20*, 81–93. [\[CrossRef\]](#)
 22. Loganovsky, K.; Perchuk, I.; Marazziti, D. Workers on Transformation of the Shelter Object of the Chernobyl Nuclear Power Plant into an Ecologically-Safe System Show QEEG Abnormalities and Cognitive Dysfunctions: A Follow-up Study. *World J. Biol. Psychiatry* **2016**, *17*, 600–607. [\[CrossRef\]](#) [\[PubMed\]](#)
 23. Barazzuol, L.; Hopkins, S.R.; Ju, L.; Jeggo, P.A. Distinct Response of Adult Neural Stem Cells to Low Versus High Dose Ionising Radiation. *Dna Repair* **2019**, *76*, 70–75. [\[CrossRef\]](#) [\[PubMed\]](#)
 24. Sato, Y.; Shinjyo, N.; Sato, M.; Osato, K.; Zhu, C.; Pekna, M.; Kuhn, H.; Blomgren, K. Grafting of Neural Stem and Progenitor Cells to the Hippocampus of Young, Irradiated Mice Causes Gliosis and Disrupts the Granule Cell Layer. *Cell Death Dis.* **2013**, *4*, e591. [\[CrossRef\]](#)
 25. Monje, M.L.; Mizumatsu, S.; Fike, J.R.; Palmer, T.D. Irradiation Induces Neural Precursor-Cell Dysfunction. *Nat. Med.* **2002**, *8*, 955–962. [\[CrossRef\]](#) [\[PubMed\]](#)
 26. Achanta, P.; Capilla-Gonzalez, V.; Purger, D.; Reyes, J.; Sailor, K.; Song, H.; Garcia-Verdugo, J.M.; Gonzalez-Perez, O.; Ford, E.; Quinones-Hinojosa, A. Subventricular Zone Localized Irradiation Affects the Generation of Proliferating Neural Precursor Cells and the Migration of Neuroblasts. *Stem Cells* **2012**, *30*, 2548–2560. [\[CrossRef\]](#) [\[PubMed\]](#)
 27. Ford, E.; Achanta, P.; Purger, D.; Armour, M.; Reyes, J.; Fong, J.; Kleinberg, L.; Redmond, K.; Wong, J.; Jang, M. Localized CT-Guided Irradiation Inhibits Neurogenesis in Specific Regions of the Adult Mouse Brain. *Radiat. Res.* **2011**, *175*, 774–783. [\[CrossRef\]](#) [\[PubMed\]](#)

28. Panagiotakos, G.; Alshamy, G.; Chan, B.; Abrams, R.; Greenberg, E.; Saxena, A.; Bradbury, M.; Edgar, M.; Gutin, P.; Tabar, V. Long-Term Impact of Radiation on the Stem Cell and Oligodendrocyte Precursors in the Brain. *PLoS ONE* **2007**, *2*, e588. [[CrossRef](#)]
29. Azizova, T.V.; Bannikova, M.V.; Grigoryeva, E.S.; Rybkina, V.L.; Hamada, N. Occupational Exposure to Chronic Ionizing Radiation Increases Risk of Parkinson's Disease Incidence in Russian Mayak Workers. *Int. J. Epidemiol.* **2020**, *49*, 435–447. [[CrossRef](#)] [[PubMed](#)]
30. Lowe, X.R.; Bhattacharya, S.; Marchetti, F.; Wyrobek, A.J. Early Brain Response to Low-Dose Radiation Exposure Involves Molecular Networks and Pathways Associated with Cognitive Functions, Advanced Aging and Alzheimer's Disease. *Radiat. Res.* **2009**, *171*, 53–65. [[CrossRef](#)]
31. Yin, E.; Nelson, D.; Coleman, M.A.; Peterson, L.E.; Wyrobek, A. Gene Expression Changes in Mouse Brain after Exposure to Low-Dose Ionizing Radiation. *Int. J. Radiat. Biol.* **2003**, *79*, 759–775. [[CrossRef](#)]
32. Gillies, M.; Richardson, D.B.; Cardis, E.; Daniels, R.D.; O'Hagan, J.A.; Haylock, R.; Laurier, D.; Leuraud, K.; Moissonnier, M.; Schubauer-Berigan, M.K.; et al. Mortality from Circulatory Diseases and Other Non-Cancer Outcomes among Nuclear Workers in France, the United Kingdom and the United States (INWORKS). *Radiat. Res.* **2017**, *188*, 276–290. [[CrossRef](#)] [[PubMed](#)]
33. Pasqual, E.; Boussin, F.; Bazyka, D.; Nordenskjold, A.; Yamada, M.; Ozasa, K.; Pazzaglia, S.; Roy, L.; Thierry-Chef, I.; de Vathaire, F.; et al. Cognitive Effects of Low Dose of Ionizing Radiation—Lessons Learned and Research Gaps from Epidemiological and Biological Studies. *Environ. Int.* **2021**, *147*, 106295. [[CrossRef](#)] [[PubMed](#)]
34. Boice, J.D.; Quinn, B.; Al-Nabulsi, I.; Ansari, A.; Blake, P.K.; Blattnig, S.R.; Caffrey, E.A.; Cohen, S.S.; Golden, A.P.; Held, K.D.; et al. A Million Persons, a Million Dreams: A Vision for a National Center of Radiation Epidemiology and Biology. *Int. J. Radiat. Biol.* **2022**, *98*, 795–821. [[CrossRef](#)] [[PubMed](#)]
35. Maximino, C.; van der Staay, F.J. Behavioral Models in Psychopathology: Epistemic and Semantic Considerations. *Behav. Brain Funct.* **2019**, *15*, 1–11. [[CrossRef](#)] [[PubMed](#)]
36. Bailey, J.; Oliveri, A.; Levin, E.D. Zebrafish Model Systems for Developmental Neurobehavioral Toxicology. *Birth Defects Res. Part C Embryo Today Rev.* **2013**, *99*, 14–23. [[CrossRef](#)] [[PubMed](#)]
37. Mommsen, T.P.; Vijayan, M.M.; Moon, T.W. Cortisol in Teleosts: Dynamics, Mechanisms of Action, and Metabolic Regulation. *Rev. Fish Biol. Fish.* **1999**, *9*, 211–268. [[CrossRef](#)]
38. Amico, J.; Mantella, R.; Vollmer, R.; Li, X. Anxiety and Stress Responses in Female Oxytocin Deficient Mice. *J. Neuroendocrinol.* **2004**, *16*, 319–324. [[CrossRef](#)]
39. Torner, L.; Plotsky, P.M.; Neumann, I.D.; de Jong, T.R. Forced Swimming-Induced Oxytocin Release into Blood and Brain: Effects of Adrenalectomy and Corticosterone Treatment. *Psychoneuroendocrinology* **2017**, *77*, 165–174. [[CrossRef](#)]
40. Harvey, B.H.; Regenass, W.; Dreyer, W.; Möller, M. Social Isolation Rearing-Induced Anxiety and Response to Agomelatine in Male and Female Rats: Role of Corticosterone, Oxytocin, and Vasopressin. *J. Psychopharmacol.* **2019**, *33*, 640–646. [[CrossRef](#)]
41. Yoshida, M.; Takayanagi, Y.; Inoue, K.; Kimura, T.; Young, L.J.; Onaka, T.; Nishimori, K. Evidence That Oxytocin Exerts Anxiolytic Effects via Oxytocin Receptor Expressed in Serotonergic Neurons in Mice. *J. Neurosci.* **2009**, *29*, 2259–2271. [[CrossRef](#)]
42. de la Mora, M.P.; Pérez-Carrera, D.; Crespo-Ramírez, M.; Tarakanov, A.; Fuxe, K.; Borroto-Escuela, D.O. Signaling in Dopamine D2 Receptor-Oxytocin Receptor Heterocomplexes and Its Relevance for the Anxiolytic Effects of Dopamine and Oxytocin Interactions in the Amygdala of the Rat. *Biochim. Biophys. Acta BBA-Mol. Basis Dis.* **2016**, *1862*, 2075–2085. [[CrossRef](#)] [[PubMed](#)]
43. Steinman, M.Q.; Duque-Wilckens, N.; Trainor, B.C. Complementary Neural Circuits for Divergent Effects of Oxytocin: Social Approach versus Social Anxiety. *Biol. Psychiatry* **2019**, *85*, 792–801. [[CrossRef](#)] [[PubMed](#)]
44. Estes, M.K.; Freels, T.G.; Prater, W.T.; Lester, D.B. Systemic Oxytocin Administration Alters Mesolimbic Dopamine Release in Mice. *Neuroscience* **2019**, *408*, 226–238. [[CrossRef](#)]
45. Baskerville, T.A.; Douglas, A.J. Dopamine and Oxytocin Interactions Underlying Behaviors: Potential Contributions to Behavioral Disorders. *CNS Neurosci. Ther.* **2010**, *16*, e92–e123. [[CrossRef](#)]
46. Dölen, G.; Darvishzadeh, A.; Huang, K.W.; Malenka, R.C. Social Reward Requires Coordinated Activity of Nucleus Accumbens Oxytocin and Serotonin. *Nature* **2013**, *501*, 179–184. [[CrossRef](#)] [[PubMed](#)]
47. Mottollese, R.; Redouté, J.; Costes, N.; Le Bars, D.; Sirigu, A. Switching Brain Serotonin with Oxytocin. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 8637–8642. [[CrossRef](#)]
48. Gutiérrez, H.C.; Colanesi, S.; Cooper, B.; Reichmann, F.; Young, A.M.J.; Kelsh, R.N.; Norton, W.H.J. Endothelin Neurotransmitter Signaling Controls Zebrafish Social Behavior. *Sci. Rep.* **2019**, *9*, 3040. [[CrossRef](#)]
49. Landin, J.; Hovey, D.; Xu, B.; Lagman, D.; Zettergren, A.; Larhammar, D.; Kettunen, P.; Westberg, L. Oxytocin Receptors Regulate Social Preference in Zebrafish. *Sci. Rep.* **2020**, *10*, 5435. [[CrossRef](#)]
50. Chuang, H.-J.; Chang, C.-Y.; Ho, H.-P.; Chou, M.-Y. Oxytocin Signaling Acts as a Marker for Environmental Stressors in Zebrafish. *Int. J. Mol. Sci.* **2021**, *22*, 7459. [[CrossRef](#)] [[PubMed](#)]
51. Gemmer, A.; Mirkes, K.; Anneser, L.; Eilers, T.; Kibat, C.; Mathuru, A.; Ryu, S.; Schuman, E. Oxytocin Receptors Influence the Development and Maintenance of Social Behavior in Zebrafish (*Danio rerio*). *Sci. Rep.* **2022**, *12*, 4322. [[CrossRef](#)]
52. Orger, M.B.; de Polavieja, G.G. Zebrafish Behavior: Opportunities and Challenges. *Annu. Rev. Neurosci.* **2017**, *40*, 125–147. [[CrossRef](#)] [[PubMed](#)]
53. Norton, W.; Bally-Cuif, L. Adult Zebrafish as a Model Organism for Behavioral Genetics. *BMC Neurosci.* **2010**, *11*, 90. [[CrossRef](#)]

- [PubMed]
54. Andersson, P.; Garnier-Laplace, J.; Beresford, N.A.; Copplestone, D.; Howard, B.J.; Howe, P.; Oughton, D.; Whitehouse, P. Protection of the Environment from Ionising Radiation in a Regulatory Context (PROTECT): Proposed Numerical Benchmark Values. *J. Environ. Radioact.* **2009**, *100*, 1100–1108. [CrossRef] [PubMed]
 55. Lerebours, A.; Gudkov, D.; Nagorskaya, L.; Kaglyan, A.; Rizewski, V.; Leshchenko, A.; Bailey, E.H.; Bakir, A.; Ovsyanikova, S.; Laptev, G. Impact of Environmental Radiation on the Health and Reproductive Status of Fish from Chernobyl. *Environ. Sci. Technol.* **2018**, *52*, 9442–9450. [CrossRef]
 56. *Help Ethovision—Version 16XT—Noldus*; Noldus Information Technology BV: Wageningen, The Netherlands, 2021; p. 945.
 57. Fontana, B.D.; Müller, T.E.; Cleal, M.; de Abreu, M.S.; Norton, W.H.J.; Demin, K.A.; Amstislavskaya, T.G.; Petersen, E.V.; Kalueff, A.V.; Parker, M.O.; et al. Using Zebrafish (*Danio rerio*) Models to Understand the Critical Role of Social Interactions in Mental Health and Wellbeing. *Prog. Neurobiol.* **2022**, *208*, 101993. [CrossRef] [PubMed]
 58. Ogi, A.; Licitra, R.; Naef, V.; Marchese, M.; Fronte, B.; Gazzano, A.; Santorelli, F.M. Social Preference Tests in Zebrafish: A Systematic Review. *Front. Vet. Sci.* **2021**, *7*, 590057. [CrossRef]
 59. Stewart, A.; Gaikwad, S.; Kyzar, E.; Green, J.; Roth, A.; Kalueff, A.V. Modeling Anxiety Using Adult Zebrafish: A Conceptual Review. *Neuropharmacology* **2012**, *62*, 135–143. [CrossRef]
 60. Pham, M.; Raymond, J.; Hester, J.; Kyzar, E.; Gaikwad, S.; Bruce, I.; Fryar, C.; Chanin, S.; Enriquez, J.; Bagawandoss, S. Assessing Social Behavior Phenotypes in Adult Zebrafish: Shoaling, Social Preference, and Mirror Biting Tests. In *Zebrafish Protocols for Neurobehavioral Research*; Springer: Berlin/Heidelberg, Germany, 2012; pp. 231–246.
 61. Ellman, G.L.; Courtney, K.D.; Andres, V., Jr.; Featherstone, R.M. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95. [CrossRef]
 62. El Houdigui, S.M.; Adam-Guillermin, C.; Loro, G.; Arcanjo, C.; Frelon, S.; Floriani, M.; Dubourg, N.; Baudalet, E.; Audebert, S.; Camoin, L. A Systems Biology Approach Reveals Neuronal and Muscle Developmental Defects after Chronic Exposure to Ionising Radiation in Zebrafish. *Sci. Rep.* **2019**, *9*, 20241. [CrossRef]
 63. Benjamini, Y.; Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B Methodol.* **1995**, *57*, 289–300. [CrossRef]
 64. Durinck, S.; Spellman, P.T.; Birney, E.; Huber, W. Mapping Identifiers for the Integration of Genomic Datasets with the R/Bioconductor Package Biomart. *Nat. Protoc.* **2009**, *4*, 1184–1191. [CrossRef] [PubMed]
 65. Armant, O.; Gombeau, K.; El Houdigui, S.M.; Floriani, M.; Camilleri, V.; Cavalie, I.; Adam-Guillermin, C. Zebrafish Exposure to Environmentally Relevant Concentration of Depleted Uranium Impairs Progeny Development at the Molecular and Histological Levels. *PLoS ONE* **2017**, *12*, e0177932. [CrossRef] [PubMed]
 66. El Houdigui, S.M.; Adam-Guillermin, C.; Armant, O. Ionising Radiation Induces Promoter DNA Hypomethylation and Perturbs Transcriptional Activity of Genes Involved in Morphogenesis during Gastrulation in Zebrafish. *Int. J. Mol. Sci.* **2020**, *21*, 4014. [CrossRef] [PubMed]
 67. Thisse, B.; Thisse, C. Protocol for In Situ Hybridization on whole Mount Zebrafish Embryos. ZFIN Database. Available online: <https://zfin.org/ZFIN/Methods/ThisseProtocol.html> (accessed on 29 July 2022).
 68. Genario, R.; Giacomini, A.C.V.V.; de Abreu, M.S.; Marcon, L.; Demin, K.A.; Kalueff, A.V. Sex Differences in Adult Zebrafish Anxiolytic-Like Responses to Diazepam and Melatonin. *Neurosci. Lett.* **2020**, *714*, 134548. [CrossRef] [PubMed]
 69. Volgin, A.D.; Yakovlev, O.A.; Demin, K.A.; de Abreu, M.S.; Alekseeva, P.A.; Friend, A.J.; Lakstygala, A.M.; Amstislavskaya, T.G.; Bao, W.; Song, C.; et al. Zebrafish Models for Personalized Psychiatry: Insights from Individual, Strain and Sex Differences, and Modeling Gene X Environment Interactions. *J. Neurosci. Res.* **2019**, *97*, 402–413. [CrossRef] [PubMed]
 70. Mineur, Y.S.; Obayemi, A.; Wigstrand, M.B.; Fote, G.M.; Calarco, C.A.; Li, A.M.; Picciotto, M.R. Cholinergic Signaling in the Hippocampus Regulates Social Stress Resilience and Anxiety-and Depression-Like Behavior. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 3573–3578. [CrossRef] [PubMed]
 71. Schmidel, A.J.; Assmann, K.L.; Werlang, C.C.; Bertoncello, K.T.; Francescon, F.; Rambo, C.L.; Beltrame, G.M.; Calegari, D.; Batista, C.B.; Blaser, R.E. Subchronic Atrazine Exposure Changes Defensive Behavior Profile and Disrupts Brain Acetylcholinesterase Activity of Zebrafish. *Neurotoxicol. Teratol.* **2014**, *44*, 62–69. [CrossRef] [PubMed]
 72. Aponte, A.; Petrunich-Rutherford, M.L. Acute Net Stress of Young Adult Zebrafish (*Danio rerio*) Is Not Sufficient to Increase Anxiety-Like Behavior and Whole-Body Cortisol. *PeerJ* **2019**, *7*, e7469. [CrossRef]
 73. Shainer, I.; Michel, M.; Marquart, G.D.; Bhandiwad, A.A.; Zmora, N.; Livne, Z.B.-M.; Zohar, Y.; Hazak, A.; Mazon, Y.; Förster, D. Agouti-Related Protein 2 Is a New Player in the Teleost Stress Response System. *Curr. Biol.* **2019**, *29*, 2009–2019. [CrossRef]
 74. Zhang, Q.; Ye, D.; Wang, H.; Wang, Y.; Hu, W.; Sun, Y. Zebrafish Cyp11c1 Knockout Reveals the Roles of 11-Ketotestosterone and Cortisol in Sexual Development and Reproduction. *Endocrinology* **2020**, *161*, bqaa048. [CrossRef]
 75. Jeanneteau, F.; Barrère, C.; Vos, M.; De Vries, C.J.; Rouillard, C.; Levesque, D.; Dromard, Y.; Moisan, M.-P.; Duric, V.; Franklin, T.C. The Stress-Induced Transcription Factor NR4A1 Adjusts Mitochondrial Function and Synapse Number in Prefrontal Cortex. *J. Neurosci.* **2018**, *38*, 1335–1350. [CrossRef] [PubMed]
 76. Shen, Y.; Raymond, P.A. Zebrafish Cone-Rod (Crx) Homeobox Gene Promotes Retinogenesis. *Dev. Biol.* **2004**, *269*, 237–251. [CrossRef] [PubMed]

- 77.
78. Braida, D.; Donzelli, A.; Martucci, R.; Capurro, V.; Busnelli, M.; Chini, B.; Sala, M. Neurohypophyseal Hormones Manipulation Modulate Social and Anxiety-Related Behavior in Zebrafish. *Psychopharmacology* **2012**, *220*, 319–330. [[CrossRef](#)] [[PubMed](#)]
79. Schmidt, R.; Strähle, U.; Scholpp, S. Neurogenesis in Zebrafish—From Embryo to Adult. *Neural. Dev.* **2013**, *8*, 3. [[CrossRef](#)]
80. Christoffel, D.J.; Golden, S.A.; Russo, S.J. Structural and Synaptic Plasticity in Stress-Related Disorders. *Rev. Neurosci.* **2011**, *22*, 535–549. [[CrossRef](#)]
81. Krężel, W.; Dupont, S.; Krust, A.; Chambon, P.; Chapman, P.F. Increased Anxiety and Synaptic Plasticity in Estrogen Receptor β -Deficient Mice. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 12278–12282. [[CrossRef](#)]
82. Kempf, S.J.; Casciati, A.; Buratovic, S.; Janik, D.; von Toerne, C.; Ueffing, M.; Neff, F.; Moertl, S.; Stenerlöv, B.; Saran, A.; et al. The Cognitive Defects of Neonatally Irradiated Mice Are Accompanied by Changed Synaptic Plasticity, Adult Neurogenesis and Neuroinflammation. *Mol. Neurodegener.* **2014**, *9*, 57. [[CrossRef](#)]
83. Hladik, D.; Tapio, S. Effects of Ionizing Radiation on the Mammalian Brain. *Mutat. Res. Rev. Mutat. Res.* **2016**, *770*, 219–230. [[CrossRef](#)]
84. Chen, Z.; Cao, K.; Xia, Y.; Li, Y.; Hou, Y.; Wang, L.; Li, L.; Chang, L.; Li, W. Cellular Senescence in Ionizing Radiation. *Oncol. Rep.* **2019**, *42*, 883–894. [[CrossRef](#)] [[PubMed](#)]
85. Kim, J.-S.; Lee, H.-J.; Kim, J.C.; Kang, S.S.; Bae, C.-S.; Shin, T.; Jin, J.-K.; Kim, S.H.; Wang, H.; Moon, C. Transient Impairment of Hippocampus-Dependent Learning and Memory in Relatively Low-Dose of Acute Radiation Syndrome Is Associated with Inhibition of Hippocampal Neurogenesis. *J. Radiat. Res.* **2008**, *49*, 517–526. [[CrossRef](#)]
86. Foray, N.; Bourguignon, M.; Hamada, N. Individual Response to Ionizing Radiation. *Mutat. Res. Rev. Mutat. Res.* **2016**, *770*, 369–386. [[CrossRef](#)] [[PubMed](#)]
87. Manda, K.; Anzai, K.; Kumari, S.; Bhatia, A. Melatonin Attenuates Radiation-Induced Learning Deficit and Brain Oxidative Stress in Mice. *Acta Neurobiol. Exp.* **2007**, *67*, 63.
88. Koc, U.; Cam, I. Chapter 24—Radiation and Oxidative Stress. In *Toxicology*; Patel, V.B., Preedy, V.R., Eds.; Academic Press: Cambridge, MA, USA, 2021; pp. 233–241. ISBN 978-0-12-819092-0.
89. Barbosa, J.S.; Ninkovic, J. Adult Neural Stem Cell Behavior Underlying Constitutive and Restorative Neurogenesis in Zebrafish. *Neurogenesis* **2016**, *3*, e1148101. [[CrossRef](#)]
90. Kizil, C.; Kaslin, J.; Kroehne, V.; Brand, M. Adult Neurogenesis and Brain Regeneration in Zebrafish. *Dev. Neurobiol.* **2012**, *72*, 429–461. [[CrossRef](#)]
91. Than-Trong, E.; Bally-Cuif, L. Radial Glia and Neural Progenitors in the Adult Zebrafish Central Nervous System. *Glia* **2015**, *63*, 1406–1428. [[CrossRef](#)] [[PubMed](#)]
92. Chen, S.; Wang, Q.-L.; Nie, Z.; Sun, H.; Lennon, G.; Copeland, N.G.; Gilbert, D.J.; Jenkins, N.A.; Zack, D.J. Crx, a Novel Otx-like Paired-Homeodomain Protein, Binds to and Transactivates Photoreceptor Cell-Specific Genes. *Neuron* **1997**, *19*, 1017–1030. [[CrossRef](#)]
93. York, J.M.; Blevins, N.A.; Meling, D.D.; Peterlin, M.B.; Gridley, D.S.; Cengel, K.A.; Freund, G.G. The Biobehavioral and Neuroimmune Impact of Low-Dose Ionizing Radiation. *Brain Behav. Immun.* **2012**, *26*, 218–227. [[CrossRef](#)] [[PubMed](#)]
94. Ung, M.-C.; Garrett, L.; Dalke, C.; Leitner, V.; Dragosa, D.; Hladik, D.; Neff, F.; Wagner, F.; Zitzelsberger, H.; Miller, G.; et al. Dose-Dependent Long-Term Effects of a Single Radiation Event on Behavior and Glial Cells. *Int. J. Radiat. Biol.* **2021**, *97*, 156–169. [[CrossRef](#)] [[PubMed](#)]
95. Gagnaire, B.; Cavalié, I.; Pereira, S.; Florian, M.; Dubourg, N.; Camilleri, V.; Adam-Guillermín, C. External Gamma Irradiation-Induced Effects in Early-Life Stages of Zebrafish, *Danio rerio*. *Aquat. Toxicol.* **2015**, *169*, 69–78. [[CrossRef](#)] [[PubMed](#)]
96. Cachat, J.; Stewart, A.; Grossman, L.; Gaikwad, S.; Kadri, F.; Chung, K.M.; Wu, N.; Wong, K.; Roy, S.; Suci, C. Measuring Behavioral and Endocrine Responses to Novelty Stress in Adult Zebrafish. *Nat. Protoc.* **2010**, *5*, 1786–1799. [[CrossRef](#)]
97. Moisan, M.-P.; Castanon, N. Emerging Role of Corticosteroid-Binding Globulin in Glucocorticoid-Driven Metabolic Disorders. *Front. Endocrinol.* **2016**, *7*, 160. [[CrossRef](#)] [[PubMed](#)]
98. Cruz, S.A.; Chao, P.-L.; Hwang, P.-P. Cortisol Promotes Differentiation of Epidermal Ionocytes through Foxi3 Transcription Factors in Zebrafish (*Danio rerio*). *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2013**, *164*, 249–257. [[CrossRef](#)] [[PubMed](#)]
99. Nguyen, M.; Yang, E.; Neelkantan, N.; Mikhaylova, A.; Arnold, R.; Poudel, M.K.; Stewart, A.M.; Kalueff, A.V. Developing ‘Integrative’ Zebrafish Models of Behavioral and Metabolic Disorders. *Behav. Brain Res.* **2013**, *256*, 172–187. [[CrossRef](#)]
100. Nozari, A.; Do, S.; Trudeau, V.L. Applications of the SR4G Transgenic Zebrafish Line for Biomonitoring of Stress-Disrupting Compounds: A Proof-of-Concept Study. *Front. Endocrinol.* **2021**, *12*, 727777. [[CrossRef](#)] [[PubMed](#)]
101. Weger, M.; Diotel, N.; Weger, B.D.; Beil, T.; Zaucker, A.; Eachus, H.L.; Oakes, J.A.; do Rego, J.L.; Storbeck, K.; Gut, P. Expression and Activity Profiling of the Steroidogenic Enzymes of Glucocorticoid Biosynthesis and the Fdx1 Co—Factors in Zebrafish. *J. Neuroendocrinol.* **2018**, *30*, e12586. [[CrossRef](#)] [[PubMed](#)]
102. Sarasamma, S.; Audira, G.; Siregar, P.; Malhotra, N.; Lai, Y.-H.; Liang, S.-T.; Chen, J.-R.; Chen, K.H.-C.; Hsiao, C.-D. Nanoplastics Cause Neurobehavioral Impairments, Reproductive and Oxidative Damages, and Biomarker Responses in Zebrafish: Throwing up Alarms of Wide

- Spread Health Risk of Exposure. *Int. J. Mol. Sci.* **2020**, *21*, 1410. [[CrossRef](#)] [[PubMed](#)]
103. Boonstra, R.; Manzon, R.G.; Mihok, S.; Helson, J.E. Hormetic Effects of Gamma Radiation on the Stress Axis of Natural Populations of Meadow Voles (*Microtus pennsylvanicus*). *Environ. Toxicol. Chem.* **2005**, *24*, 334–343. [[CrossRef](#)] [[PubMed](#)]
104. Nagpal, J.; Herget, U.; Choi, M.K.; Ryu, S. Anatomy, Development, and Plasticity of the Neurosecretory Hypothalamus in Zebrafish. *Cell Tissue Res.* **2019**, *375*, 5–22. [[CrossRef](#)]
105. Amer, S.; Brown, J.A. Glomerular Actions of Arginine Vasotocin in the In Situ Perfused Trout Kidney. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **1995**, *269*, R775–R780. [[CrossRef](#)]
106. Chan, D.K. Comparative Physiology of the Vasomotor Effects of Neurohypophysial Peptides in the Vertebrates. *Am. Zool.* **1977**, *17*, 751–761. [[CrossRef](#)]
107. Feldman, R.; Monakhov, M.; Pratt, M.; Ebstein, R.P. Oxytocin Pathway Genes: Evolutionary Ancient System Impacting on Human Affiliation, Sociality, and Psychopathology. *Biol. Psychiatry* **2016**, *79*, 174–184. [[CrossRef](#)] [[PubMed](#)]
108. Steinman, M.Q.; Duque-Wilckens, N.; Greenberg, G.D.; Hao, R.; Campi, K.L.; Laredo, S.A.; Laman-Maharg, A.; Manning, C.E.; Doig, I.E.; Lopez, E.M. Sex-Specific Effects of Stress on Oxytocin Neurons Correspond with Responses to Intranasal Oxytocin. *Biol. Psychiatry* **2016**, *80*, 406–414. [[CrossRef](#)] [[PubMed](#)]
109. Zheng, J.; Babygirija, R.; Bülbül, M.; Cerjak, D.; Ludwig, K.; Takahashi, T. Hypothalamic Oxytocin Mediates Adaptation Mechanism against Chronic Stress in Rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2010**, *299*, G946–G953. [[CrossRef](#)] [[PubMed](#)]
110. Ferguson, J.N.; Aldag, J.M.; Insel, T.R.; Young, L.J. Oxytocin in the Medial Amygdala Is Essential for Social Recognition in the Mouse. *J. Neurosci.* **2001**, *21*, 8278–8285. [[CrossRef](#)]
111. Kirsch, P.; Esslinger, C.; Chen, Q.; Mier, D.; Lis, S.; Siddhanti, S.; Gruppe, H.; Mattay, V.S.; Gallhofer, B.; Meyer-Lindenberg, A. Oxytocin Modulates Neural Circuitry for Social Cognition and Fear in Humans. *J. Neurosci.* **2005**, *25*, 11489–11493. [[CrossRef](#)]
112. Goodson, J.L.; Schrock, S.E.; Klatt, J.D.; Kabelik, D.; Kingsbury, M.A. Mesotocin and Nonapeptide Receptors Promote Estrildid Flocking Behavior. *Science* **2009**, *325*, 862–866. [[CrossRef](#)] [[PubMed](#)]
113. Nonnis, S.; Angiulli, E.; Maffioli, E.; Frabetti, F.; Negri, A.; Cioni, C.; Alleva, E.; Romeo, V.; Tedeschi, G.; Toni, M. Acute Environmental Temperature Variation Affects Brain Protein Expression, Anxiety and Explorative Behavior in Adult Zebrafish. *Sci. Rep.* **2021**, *11*, 2521. [[CrossRef](#)]
114. Nunes, A.R.; Gliksberg, M.; Varela, S.A.; Teles, M.; Wircer, E.; Blechman, J.; Petri, G.; Levkowitz, G.; Oliveira, R.F. Developmental Effects of Oxytocin Neurons on Social Affiliation and Processing of Social Information. *J. Neurosci.* **2021**, *41*, 8742–8760. [[CrossRef](#)]
115. Onaka, T.; Takayanagi, Y. Role of Oxytocin in the Control of Stress and Food Intake. *J. Neuroendocrinol.* **2019**, *31*, e12700. [[CrossRef](#)]
116. Gilchrist, B.; Tipping, D.; Hake, L.; Levy, A.; Baker, B. The Effects of Acute and Chronic Stresses on Vasotocin Gene Transcripts in the Brain of the Rainbow Trout (*Oncorhynchus mykiss*). *J. Neuroendocrinol.* **2000**, *12*, 795–801. [[CrossRef](#)]
117. Kulczykowska, E. Responses of Circulating Arginine Vasotocin, Isotocin, and Melatonin to Osmotic and Disturbance Stress in Rainbow Trout (*Oncorhynchus mykiss*). *Fish Physiol. Biochem.* **2001**, *24*, 201–206. [[CrossRef](#)]
118. Smith, C.J.; DiBenedictis, B.T.; Veenema, A.H. Comparing Vasopressin and Oxytocin Fiber and Receptor Density Patterns in the Social Behavior Neural Network: Implications for Cross-System Signaling. *Front. Neuroendocrinol.* **2019**, *53*, 100737. [[CrossRef](#)] [[PubMed](#)]
119. Song, Z.; Albers, H.E. Cross-Talk among Oxytocin and Arginine-Vasopressin Receptors: Relevance for Basic and Clinical Studies of the Brain and Periphery. *Front. Neuroendocrinol.* **2018**, *51*, 14–24. [[CrossRef](#)] [[PubMed](#)]
120. Ribeiro, D.; Nunes, A.R.; Gliksberg, M.; Anbalagan, S.; Levkowitz, G.; Oliveira, R.F. Oxytocin Receptor Signaling Modulates Novelty Recognition but Not Social Preference in Zebrafish. *J. Neuroendocrinol.* **2020**, *32*, e12834. [[CrossRef](#)] [[PubMed](#)]
121. Buske, C.; Gerlai, R. Maturation of Shoaling Behavior Is Accompanied by Changes in the Dopaminergic and Serotonergic Systems in Zebrafish. *Dev. Psychobiol.* **2012**, *54*, 28–35. [[CrossRef](#)] [[PubMed](#)]
122. Lanfumey, L.; Mongeau, R.; Cohen-Salmon, C.; Hamon, M. Corticosteroid–Serotonin Interactions in the Neurobiological Mechanisms of Stress-Related Disorders. *Neurosci. Biobehav. Rev.* **2008**, *32*, 1174–1184. [[CrossRef](#)]
123. Maximino, C.; Puty, B.; Benzecry, R.; Araújo, J.; Lima, M.G.; Batista, E.; de, J.O.; de Matos Oliveira, K.R.; Crespo-Lopez, M.E.; Herculano, A.M. Role of Serotonin in Zebrafish (*Danio rerio*) Anxiety: Relationship with Serotonin Levels and Effect of Buspirone, WAY 100635, SB 224289, Fluoxetine and Para-Chlorophenylalanine (pCPA) in Two Behavioral Models. *Neuropharmacology* **2013**, *71*, 83–97. [[CrossRef](#)]
124. Kacprzak, V.; Patel, N.A.; Riley, E.; Yu, L.; Yeh, J.-R.J.; Zhdanova, I.V. Dopaminergic Control of Anxiety in Young and Aged Zebrafish. *Pharmacol. Biochem. Behav.* **2017**, *157*, 1–8. [[CrossRef](#)]

Supplementary information

Table S1: effective dose rates were estimated by MCNPX v2.70 (a) for behavioral tests, (b) for RNA Sequencing

Figure S1: Oxytocin expression in control and 5 mGy.h⁻¹ irradiated fish after fluorescent RNA *in situ* hybridization

Table S2: Go terms identifiers for neurohormones and neurotransmitters

Table S1

(a)

	D05 condition Dose mGy/h	Mean	Standard deviation	D5 condition Dose mGy/h	Mean	Standard deviation
tank 1	0,45	0,49	0,06	5,12	5,06	0,109877698
tank 2	0,48			5,03		
tank 3	0,53			5,15		
tank 4	0,40			4,90		
tank 5	0,54			4,97		
tank 6	0,55			5,17		

(b)

	D005 condition Dose mGy/h	Mean	Standard deviation	D5 condition Dose mGy/h	Mean	Standard deviation
tank 1	0,02	0,04	0,018473664	5,18	3,54	3,54
tank 2	0,02			4,83		
tank 3	0,02			4,73		
tank 4	0,05			2,49		
tank 5	0,05			1,82		
tank 6	0,05			2,20		

Figure S1

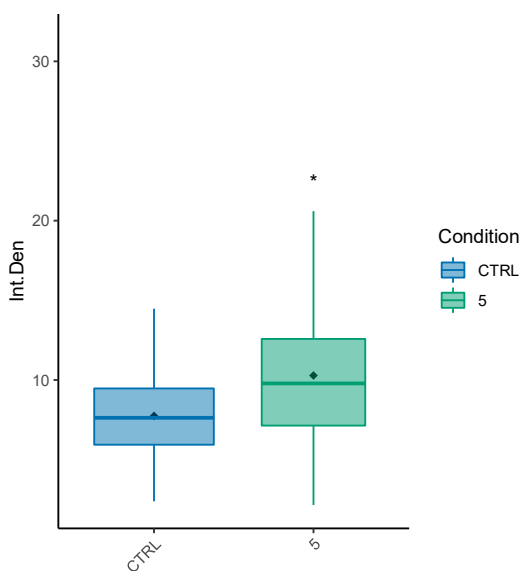


Table S2

Neurohormones/neurotransmitters	Go terms
Acetylcholine	GO:0008292, GO:0022848, GO:0015464, GO:0042166, GO:0005892, GO:0071340, GO:0016907, GO:0007197, GO:0005277, GO:0007213, GO:0033130, GO:1904395, GO:0003990, GO:0006581, GO:0030548, GO:0030550
Angiotensin	GO:0031701, GO:0038166, GO:0003081, GO:0002003, GO:0004945, GO:0001596, GO:0086097
Arginin-vasopressin	GO:0005000, GO:0001992
Corticotropin	GO:0004978, GO:0071376, GO:0043404, GO:0051424, GO:0051460, GO:1900011, GO:0051431, GO:0051429
Cortisol	GO:0051414, GO:0034650, GO:0034651
Dopamine	GO:0004968, GO:0097211, GO:0005183, GO:0031530
Epinephrin	GO:0001994, GO:0042421, GO:0032812, GO:0048242, GO:0071871, GO:0042415, GO:0005334, GO:0015874, GO:0051620
GABA	GO:0097154, GO:0004890, GO:0022851, GO:0051932, GO:1902711, GO:0032228, GO:0004965, GO:0050811
Glucocorticoid	GO:0051384, GO:0004883, GO:0042921, GO:0043402, GO:0006704, GO:0035259, GO:0031943, GO:0071385
Glutamate	GO:0004970, GO:0008066, GO:0035235, GO:0035249, GO:0004971, GO:0032281, GO:0098978, GO:0004972, GO:0017146, GO:0008328, GO:0015277, GO:0032983, GO:0007216, GO:0051966, GO:0035256, GO:0001641, GO:0007196, GO:0051967, GO:2000312, GO:0035255, GO:0051968, GO:0098990, GO:1905962
Gonadotropin	GO:0004968, GO:0097211, GO:0005183, GO:0031530
Hypocretin	GO:0031771, GO:0031772, GO:0042324
Melatonin	GO:0008502, GO:0030186, GO:0030187
Norepinephrine	GO:0001994, GO:0042421, GO:0042415, GO:0005334, GO:0015874, GO:0051620
Oxytocin	GO:0004990
Serotonin	GO:0004993, GO:0051378, GO:0007198, GO:0098664, GO:0005335, GO:0051610, GO:0006837, GO:0031821, GO:0042427, GO:0042428, GO:0007210
Thyrotropin	GO:0031531, GO:0004997, GO:0008437

3.1.3. Main results and discussion

AXIS I: From molecular effects to stress behavior and reduced sociability

Question 1: What are the main molecular modifications after chronic low to moderate dose rates of ionizing radiation?

We first detected a dose rate-dependent increase of differentially expressed genes (DEG) and any common gene was found with all three irradiated conditions. Since very few genes were modulated after 0.05 mGy.h^{-1} with responses that can occur with several type of stressors (chaperon-mediated autophagy, response to heat), the search for any specific marker in that condition was not pursued. Among the 156 genes that were commonly modulated between 0.5 mGy.h^{-1} and 5 mGy.h^{-1} irradiated conditions, the altered pathways included visual perception, function in synaptic signaling and activities of neurotransmitters and their receptors which is in accordance with other studies that aimed at evaluating the molecular effects after low dose of ionizing radiation. These responses vary from the impact of higher dose of IR that showed impacted pathways associated with mitochondria, protein biosynthesis and metabolism (Lowe et al., 2009). Additionally, no inflammation process, apoptosis, DNA damage or oxidative stress (at the levels of transcript), and by immunofluorescent assay of fluorojadeC (data not shown), was detected in the experimental conditions tested here. One of the documented responses of acute high doses of ionizing radiation is the reduction in neurogenesis (Achanta et al., 2012, 2009; Raber et al., 2004). Here, we observed the upregulation of several genes with known function in neuronal specification and/or differentiation (*faimb*, *insm1a*, *atf3*, *bsx*) suggestive of a positive regulation of neurogenesis. Therefore, additionally to Article 1, we analyzed a list of 895 genes involved in the process of neurogenesis (associated to GO Term GO:0022008) (**Figure 20**).

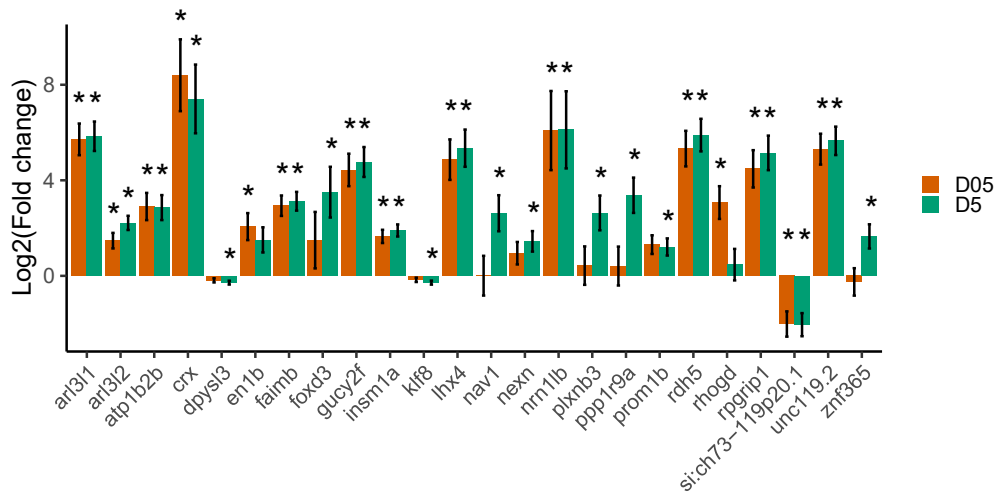


Figure 20 – Expression of genes with function in neurogenesis. *p-value <0.05

After radiation at 0.5 mGy.h⁻¹, 18 genes were dysregulated with 15 upregulated genes and 3 down regulated genes. In D5 condition, among the 23 DEGs, 20 were upregulated and 3 down regulated. This induction of neurogenesis is supported notably by the strong upregulation of *crx*, a transcription factor involved in neuronal differentiation of photoreceptors in the retina. Its role in the telencephalon is currently unknown, however we hypothesized that it might be involved in the process of neurogenesis in this area as suggested through its upregulation in the VZ of the PPa after RNA *in situ* hybridization.

Question 2: Is there any modification at the individual scale and could it be predicted by the transcriptomic analysis?

Three behavioral tests were performed, the novel tank test (NTT), the sociability preference test (SP) and the shoaling test (ShT). We first noticed the reduction in locomotion through the decrease in the total distance travelled only in female zebrafish. We then showed that this hypolocomotion state was not related to modification of acetylcholinesterase activity in the brain or muscles. We additionally detected the establishment of a stress response and a reduced individual social response only in females. We could then relate these behavioral patterns to the upregulation of genes with function in serotonin or dopamine synthesis and transport (*tph1a*, *tph2*, *slc6a2*, *slc18a2*, *ddc*), in cortisol production or secretion (*cyp11c1*, *asip2b*), the stress-induced transcription factors *nr4a1* and *nr3c2* as well as an increased expression of oxytocin and

arginine vasopressin. Hereafter, the behavioral stress response is very likely to be due to an activation of the HPI axis with increased cortisol secretion and the hypolocomotion state is presumably a consequence of increased freezing time. Additionally, oxytocin and serotonin were demonstrated to interact with HPI axis so they might play a role in the observed stress response as well as arginine vasopressin. Since the pattern of expression of oxytocin via *in situ* hybridization showed the increase of cells in the preoptic nucleus, this is interpreted as the activation of this area which is included in the social behavioral network of the social decision-making network after chronic low to moderate dose rates of ionizing radiation. Oxytocin might act through its *oxtr1* receptors for the regulation of social preference and in synergy with vasopressin system since several studies related the crosstalk between these systems. However, the expression of *oxtr1* was not modified and the activation of this receptor was not tested. Thus, we cannot rule out that a decrease in the degree of sociability could be an indirect consequence of the observed stress response. Serotonin was showed to interact with oxytocin and dopamine is intricated in the reward system of the SMDN which gives arguments towards activation of this network. Also, we cannot rule out that other brain areas are implicated in these patterns' behaviors. For example, the habenula regulates anxiety response and is also involved in the social decision-making network through connections with the preoptic area (among others). It would be of great interest to assess the role of the habenula in the response observed after exposure to chronic low to moderate low dose rates of radiations.

Herein, our results highlight a complex interaction between various neurohormones and neurotransmitters in response to stress, which together have consequences on behavior. Contrastingly to transcriptomics, it was not possible to highlight a dose-response relationship in behavioral analysis. However, some markers were identified and could be integrated in the evaluation to the response to chronic low to moderate dose rates of ionizing radiation as for example oxytocin, arginine vasopressin or serotonin. These markers could contribute to the development of radiosensitivity indicators and be employed as danger characterization useful for risk assessment of low dose rates of ionizing radiation in ecotoxicology and for humans.

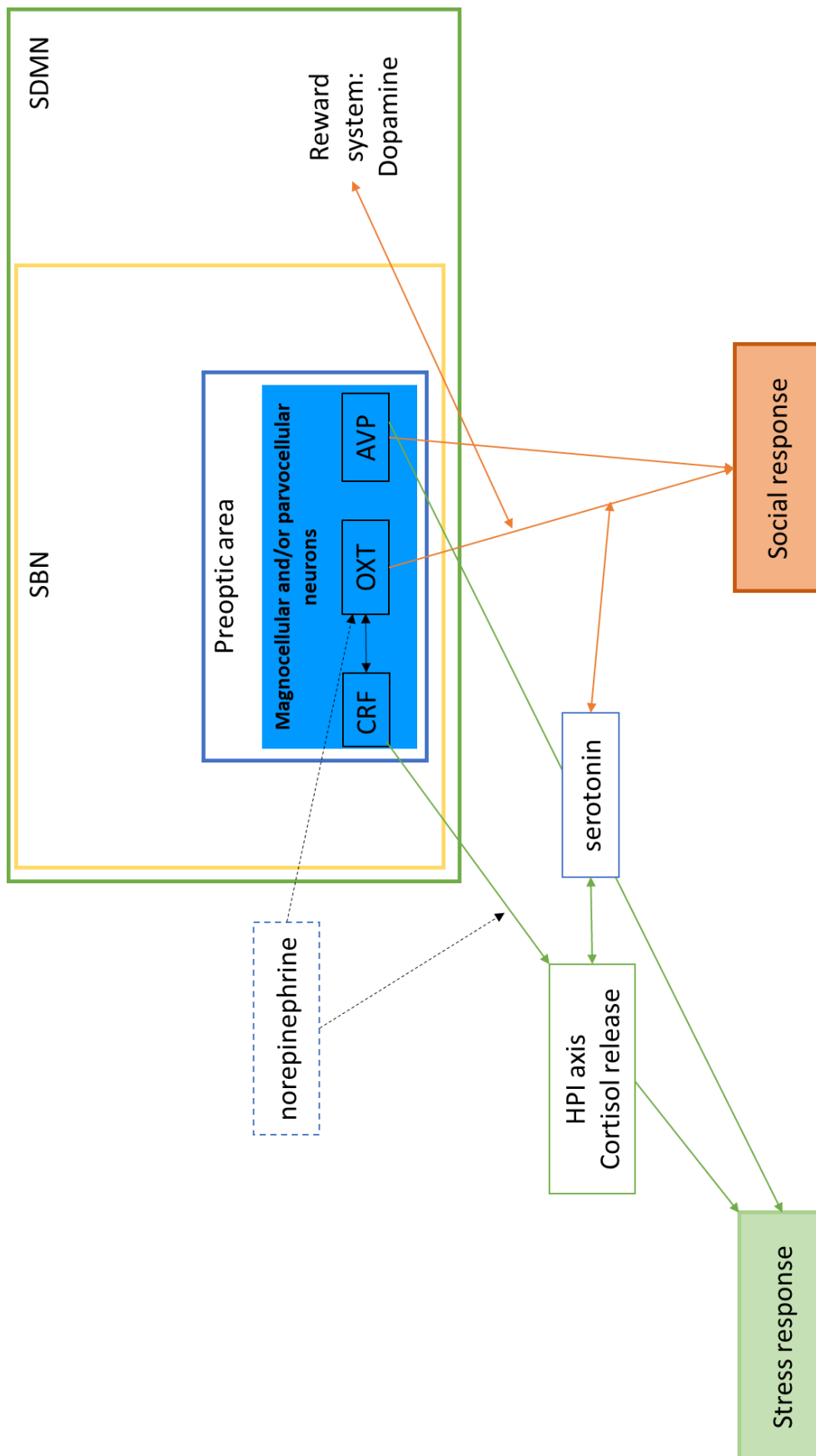


Figure 21 – Schematic overview of the molecular and behavioral effects after chronic exposure to low to moderate dose rates of IR. Arrows indicate the interactions that might act in conjunction to induce the stress response (in green) and reduce sociability (in orange). Norepinephrine constitutes an added proposition since the transporter *slc6a2* was upregulated.

3.2. Axis II: Common and differential features of IR response compared to regenerative neurogenesis induced by neuroinflammation

3.2.1. Objectives of scientific paper

The initiation of reactive neurogenesis after stabwound injury is well known to be dependent on neuroinflammation. We aimed at comparing all processes involved in regenerative neurogenesis after physical injury to better understand how neurogenesis is affected after chronic low to moderate dose rates of IR by transcriptomic analysis associated with RNA *in situ* hybridization.

3.2.2. Reactive neurogenesis is induced after chronic exposure to ionizing radiation at moderate dose rates in adult zebrafish

Reactive neurogenesis is induced after chronic exposure to ionizing radiation at moderate dose rates in adult zebrafish

Elsa Cantabella, Isabelle Cavalie, Virginie Camilleri, Nicolas Dubourg, Olivier Armant

1. Abstract

The effects of high doses of ionizing radiation (IR) on the developmental and adult brain has been extensively described over the years through the observation of microcephaly or cognitive impairments tightly linked to the reduction of neuronal progenitors. However, knowledge on the effects of chronic low to moderate dose rates of IR on adult brain is still scarce. In adult zebrafish, after continuous exposure to IR, an increased stress behavior and decrease in sociability were observed associated with modulations in the expression of the neurohormone oxytocin and the upregulation of genes involved in neurotransmission and neurogenesis in the telencephalon. Adult brain neurogenesis relies on the presence of neural stem cells (NSC) able to proliferate and to generate novel neuronal and glial cells. In addition, zebrafish NSC can be mobilized to

regenerate brain cells and function after injury through reactive neurogenesis, a process triggered by neuroinflammation. In this study we aimed at deciphering the molecular effects of IR on adult neurogenesis under chronic stress using adult zebrafish exposed continuously to gamma radiation for 36 days at 0.5 mGy.h⁻¹ and 5 mGy.h⁻¹. In addition, we assessed the potential involvement of neuroinflammation in this process, by characterizing commonalities and differences in NSC recruitment after stab wound injury. The time course of transcriptomic study of adult brain regeneration was performed at 1 hour post-lesion 1hpl, 3hpl, 10hpl, 24hpl, 96hpl and 240hpl, allowing the precise molecular profiling of the processes ongoing during the different phases of regeneration. Transcriptomics analysis after stabwound injury demonstrated the modulation of ion transport and axonogenesis, the induction of genes involved in visual perception and of the gene *atf3* involved in regeneration in the spinal cord as soon as 1hpl, revealing the potential implication of these pathways in the immediate early response after wound. Genes with function in visual perception were also induced after exposure to continuous stress. Finally, cortisol-related genes were upregulated in irradiated fish but not genes with function in inflammation. Altogether, these data highlight that regenerative neurogenesis is activated under chronic stress of ionizing radiation and point towards a novel unanticipated role of genes with function in visual perception in the initial responses to stress and injury.

2. Introduction

The zebrafish (*Danio rerio*) has become popular over the last past years as it offers ease of maintenance, fast life cycle and 70 % of zebrafish genes have orthologues in human (Howe et al., 2013) which make this animal model well suited to study brain function and complex brain disorders (Kalueff et al., 2014). Indeed, the adult brain of zebrafish contains sixteen distinct neurogenic niches, including in the telencephalon, the more rostral part of the brain. The telencephalon is subdivided in pallial and subpallial domains which share homology with the dentate gyrus of the hippocampus and the subventricular zone of mammals 'brain. Radial glial cells located in the ventricular zone constitute the adult neural stem cell population which generate functional neurons that integrate into the brain circuitry through constitutive neurogenesis throughout life (Adolf et al., 2006; Diotel et al., 2020; Schmidt et al., 2013). The zebrafish displays a high regenerative

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capacity of the adult central nervous system compared to mammals (Becker et al., 1997; Grandel et al., 2006; März et al., 2011; Raymond et al., 2006). Importantly, this process was proposed to be tightly linked to the induction of neuroinflammation, proposed to be an essential early trigger of adult regenerative neurogenesis (Alunni and Bally-Cuif, 2016; Kyritsis et al., 2012).

Computational analyses such as toxicogenomic allow to study the molecular mechanisms induced by stressors making possible the identification of early biological responses. Kinetic studies are especially relevant to understand the molecular basis of biological responses as they describe the dynamic of the response over time. Such data can be used to model biological responses and disentangle the effects of perturbation on the transcriptome dynamics. Ionizing radiation is a prototypical genotoxic stressor for which a deep scientific knowledge is available. This ranges from energy deposition of charged particles or photons on living tissues, to epidemiological studies on atomic bomb survivors in Hiroshima and Nagasaki (Folley et al., 1952). Effects of IR on brain include microcephaly, decreased cognitive performance and seizures (Dunn et al., 1990; Otake and Schull, 1998; Plummer, 1952; Schull and Otake, 1999). In addition, exposures of adult brain to high and acute dose of IR reduce the number of neuronal progenitors (Hellström et al., 2009; Lazarini et al., 2009). Emerging evidence describe neurological effects of adult exposure to radiation at dose rates much lower than previously thought in humans including elevated risk of Parkinson's disease (Azizova et al., 2020) and cerebrovascular defects (Gillies et al., 2017). The effects of IR on adult neural stem cells and proliferating neural precursor cells were demonstrated in the two adult neurogenic niches in rodents namely the subventricular zone and the hippocampus (Curtis et al., 2007; Farin et al., 2009; Moe et al., 2005; Reynolds and Weiss, 1992; Sanai et al., 2011; Wang et al., 2011). In addition, a recent study in adult zebrafish demonstrated an increased stress behavior after continuous exposure to IR (36 days) associated with the modulation of expression of the neurohormone oxytocin and the upregulation of genes involved in neurotransmission and neurogenesis in the telencephalon (Cantabella et al., 2022). However, the precise effects of chronic low to moderate dose rates of IR on adult neurogenesis remain unknown. Furthermore, the molecular mechanisms involved in the initiation and maintenance of the injury-induced regenerative response in the adult zebrafish brain remain scarce, especially on the immediate early response occurring just after wound, and it remain unclear if adult neurogenesis can be induced independently of

neuroinflammation. Consequently, in this study we aimed at deciphering the molecular effects of IR on adult neurogenesis and the potential involvement of neuroinflammation under chronic stress by comparing the response to brain regeneration after injury. The transcriptomic profiling of brain regeneration dynamic was analyzed after brain injury at 1 hour post lesion (hpl), 3 hpl, 10 hpl, 24 hpl, 96 hpl and 240 hpl and compared to moderate dose rates of exposure of ionizing radiation at $0.5 \text{ mGy}\cdot\text{h}^{-1}$ and $5 \text{ mGy}\cdot\text{h}^{-1}$ for 36 days. Furthermore, we identified genes (*atf3*, *aanat2*, *ddc*) as immediate early genes involved in the response just after wound and under chronic stress.

3. Materials and Methods

3.1. Zebrafish maintenance and irradiation

Adult zebrafish were maintained at the Institut de Radioprotection et de Sûreté Nucléaire facilities in accordance with French and European regulations on protection of animals used for scientific purposes (EC Directive 2010/63/EU and French Decret 2013–118). All experiments were approved by the ethical comity (C2EA, IRSN, France) and the French Ministry of Research under the reference APAFIS#11488. Six to nine months old wildtype zebrafish of AB strain were provided by Amagen (Gif-sur-Yvette, France) and Sorbonne Paris University (Institut de Biologie Paris Seine). Constant conditions were preserved for fish maintenance: $27 \pm 1 \text{ }^\circ\text{C}$, 350–450 $\mu\text{S}/\text{cm}$, pH 7.5 and 12/12 h dark-light cycles, in ZebTEC system (Techniplast, France). Fish were fed three times a day with Gemma Micro 600. Fish were either chronically exposed during 36 days to gamma irradiation by a ^{137}Cs source in the MICADO experimental irradiation facility (IRSN, Cadarache, France), except for a duration of one hour 5 times a week without irradiation to visually verify fish's health. For irradiation, two dose rates were employed: $0.5 \text{ mGy}\cdot\text{h}^{-1}$ and $5 \text{ mGy}\cdot\text{h}^{-1}$. To confirm dose rates of exposure, we used operational dosimetry with radio-photoluminescent dosimeters (RPL, GD-301 type, Chiyoda Technol Corporation Japan) and the effective dose rates were estimated by MCNPX v2.70 (Cantabella et al., 2022).

3.2. Stabwound injury

Stab wound injury was performed on anesthetized fish in the right side of the telencephalon, keeping the left hemisphere as an unlesioned control as described before (Schmidt et al., 2014). Briefly, fish were anesthetized by immersion in benzocaine at 168 mg/ml for about 1 min until regular ventilation of the gills. Lesion in the right hemisphere of the telencephalon was performed under the binocular by inserting an insulin needle through the skull. The wounded fish were transferred in a separate tank with clean water and monitored until awake for normal swimming behavior. Wounded fish were euthanized by hypothermic shock on ice (2-4°C) and behead 1-hour post-lesion (hpl), 3hpl, 10hpl, 24hpl, 96hpl and 240 hpl. Telencephalon were dissected under binoculars.

3.3. Library preparation and RNA sequencing.

Telencephalon were dissected under the binocular, just after euthanasia of irradiated or stab wounded fish. For irradiated samples, each sample consisted of 3 to 4 dissected telencephalons (left and right hemispheres) made in 4 to 6 replicates. For the stab-wounded fish, the wounded right hemisphere was separated from the left hemisphere which used as an unwounded control. 3 hemispheres were pooled in duplicates. Telencephalon were homogenized in 200 µl of Trizol (LifeTechnologies) using beads homogenizer (Precellys Evolution, Bertin Technologies) with 2 cycles of 30 sec each. Total RNA extraction was performed following manufacturer instructions with an additional chloroform extraction before isopropanol precipitation. RNA integrity (RIN), quality and concentration were assessed using RNA Nano Chips (Bioanalyzer 2100, Agilent). All samples had a RIN > 8. Sequencing libraries were generated from 1 µg of total RNA following the TruSeq mRNA stranded protocol (Illumina, San Diego, CA, USA). After quality check and concentration determination on DNA1000 Chips (Bioanalyzer 2100, Agilent, Les Ulis, France), libraries were run on a HiSeq4000 platform to produce 50 bases long paired end reads (GenomeEast, IGBMC, Illkirch, France).

3.4. Analysis of transcriptomics data

Quality controls of reads was made as described before (Murat El Houdigui et al., 2019). Briefly, reads quality was assessed with an in-house pipeline to determine quality over read length, duplication rates, insert size, adaptors contamination, nucleotide composition and mapping rates against the reference genome (RNA-STAR with GRCz11

and known exon–exon junctions from Ensembl release 98). Normalisation and differential expression analysis were performed with DESeq2 v1.30.1. Biological reproducibility was assessed by hierarchical clustering of the variance stabilized expression data (rlog) obtained from DESeq2 with Pearson’s correlation and complete linkage method. Genes with $|\text{fold change}| \geq 1.5$ and p-value corrected for multiple testing, $\text{fdr} < 0.05$ (false discovery rate (Benjamini and Hochberg, 1995) were considered as differentially expressed in all analysis. Afterwards, functional enrichments was performed using Gene ontology (GO) with R package clusterProfiler using zebrafish genes retrieved by Ensembl biomaRt (as published before, Armant et al., 2017; Durinck et al., 2009; Murat El Houdigui et al., 2020). Significance was considered when fdr from Fisher’s exact test was < 0.05 . To perform kinetic analysis, we focused on the DEGs of the lesioned hemisphere only and used mfuzz for clustering. Functional enrichments were produced as previously described. For the volcano-plot of differentially expressed genes (DEG) of corticotropin, cortisol or glucocorticoid, the following GO terms were used to select genes with the R package biomaRt (Durinck et al., 2009): Corticotropin (GO:0004978, GO:0071376, GO:0043404, GO:0051424, GO:0051460, GO:1900011, GO:0051431, GO:0051429), cortisol (GO:0051414, GO:0034650, GO:0034651), glucocorticoid (GO:0051384, GO:0004883, GO:0042921, GO:0043402, GO:0006704, GO:0035259, GO:0031943, GO:0071385).

3.5. RNA in situ hybridization on adult brain sections

We performed *in situ* hybridization on fish irradiated at 5 mGy.h^{-1} and on 1 hour post injury on lesioned brains. Antisense RNA probes labelled with digoxigenin (DIG) for *ddc*, *atf3* and *aanat2* were cloned by RT PCR into PCRII vector as follows. Reverse transcription of $1 \mu\text{g}$ of total RNA from adult brain was performed using SuperScript III (Life Technologies), dNTP, polydT primers, following manufacturer instructions. PCR amplification was made using Taq platinum kit (Life Technologies) with $1 \mu\text{L}$ of RT and the primers pairs: *atf3* according to Armant and colleagues (Armant et al., 2013), *aanat2* forward CTCACCCTTCCTCAAACCCT, *aanat2* reverse TCGCGTTTCAACACACACAT, *ddc* forward TCTTATCCTGCAATGCTCGC and *ddc* reverse: CTCCTGGACATGACGTGACT. Antisens DIG probe were generated from linearised vector as described before (Cantabella et al., 2022). RNA *in situ* hybridization labelling on vibratome slice was described before (Cantabella et al., 2022). 2-3 brains were used per condition. Each *in situ*

hybridization staining was repeated at least 2 times. Expression patterns were observed under a Leica binocular (DM750) equipped with a Leica ICC50 camera at x4, and x20 zoom.

4. Results

4.1. Differential expression analysis in injured telencephalon compared to uninjured telencephalon

To decipher the transcriptional mechanisms triggered during brain regeneration we induced brain injury by stabwound on one side of the adult telencephalon. The transcriptomic response was assessed by differential gene expression analysis using the contralateral telencephalic ventricle as uninjured control at 1h post -lesion (hpl), 3hpl, 10hpl, 96 hpl and 240 hpl. The number of differentially expressed genes (DEG) after time post lesion peaked at 96 hpl followed by a sharp decrease at 240 hpl. Most genes were upregulated except at 96hpl where the ratio was close to 50:50 (**Figure 1A**). To verify the quality of this dataset, we first checked for the deregulation of genes known to be modulated during regenerative neurogenesis. *Pcna*, known to promote cell proliferation was upregulated at 96 hpl (FC=0.85, fdr<10⁻⁰⁸). *Bdnf* was reported to be increased as soon as 1 day after the lesions and to strengthens the proliferative activity of neuronal precursor cells, assists the neuronal migration toward the injured areas and demonstrates survival properties due to its antiapoptotic effects (Cacialli et al., 2018). Our results showed the upregulation of *bdnf* at 3 hpl and 10 hpl (FC= 1.59, fdr< 10⁻⁰⁴ and FC= 1.42, fdr= 0.0008 respectively). *Id1* that controls the cell stem pool by keeping neural stem cells quiescent (Viales et al., 2015) was found to be upregulated at 96 hpl (FC=0.57, fdr=0.003). In early phase regeneration (one day post-lesion), *socs3* was described as highly upregulated in zebrafish cord regeneration for the control of inflammatory response acting as a suppressor of cytokine signaling (Hui et al., 2014). However, we observed the upregulation of *socs3* as soon as 1 hpl and 3 hpl in our results as well as 96 hpl (FC=1.32, fdr=0.05; FC=1.42, fdr=0.009 and FC=1.72, fdr=0.0002 respectively). *Cxcr4b* plays a role in the immune response for macrophage activation and its gene expression was increased in retinal regeneration at 2 days post lesion (Cameron et al., 2005). Here, we observed a significant upregulation at 24 hpl and 96 hpl (FC=1.52, fdr<10⁻⁰⁸ and FC=0.76, fdr=0.004 respectively) (data not shown).

We then performed Overrepresentation analysis to highlight the biological process in play at the different time post lesion. Each time post lesion was mainly characterized by its own modulated biological pathway (**Figure 1B**). At 1 hpl, many upregulated genes were involved in sensory perception of light stimulus (GO:0050953, $fdr < 10^{-14}$) or in phototransduction (GO:0007602, $fdr < 10^{-04}$), in leukocyte migration such as *il1b* or *myd88* (GO:0050900, $fdr = 0.04$) and in the regulation of cell population proliferation (GO:0042127, $fdr < 10^{-04}$). Among the downregulated genes, we observed enrichments in alpha-beta T cell activation involved in immune response (GO:0002287, $fdr = 0.04$) at 1 hpl. At 3 hpl, the regulation of protein phosphorylation (GO:0001932, $fdr = 0.04$) was observed as well as the positive regulation of protein metabolic process (GO:0051247, $fdr = 0.03$). At 10 hpl, we observed the enrichment of cellular response to growth factor stimulus with upregulated genes such as *tgfb1b* (GO:0071363, $fdr = 0.03$). Activation of the immune system process (GO:0002682, 24 hpl: $fdr < 10^{-10}$, 96 hpl: $fdr < 10^{-05}$) with lymphocyte activation (GO:0046649, 24 hpl $fdr < 10^{-04}$, 96 hpl: $fdr = 0.02$), cytokine mediated signaling pathway (GO:0019221, 24 hpl: $fdr = 0.0002$, 96 hpl: $fdr = 0.0003$), phagocytosis (GO:0006909, 24 hpl: $fdr = 0.03$) and macrophage activation (GO:0042116, 24 hpl: $fdr = 0.002$) with for example upregulation of *marco* and *spi1b* and inflammatory response (GO:0006954, 24 hpl: $fdr = 0.001$, 96 hpl: $fdr = 0.008$) were translated through 24 hpl stage and/or 96 hpl. The latter is also described by the activation of DNA replication (GO:0006260, $fdr < 10^{-13}$), mitotic cell cycle process (GO:1903047, $fdr < 10^{-21}$) and positive regulation of apoptotic process (GO:0043065, $fdr = 0.008$) (Supplementary Materials, Figure S1). In addition, regulation of neurogenesis (GO:0050767, $fdr = 0.005$), regulation of axonogenesis (GO:0050770, $fdr < 10^{-04}$) and regulation of ion transport (GO:0043269, $fdr < 10^{-12}$) at 96 hpl were enriched with downregulated genes (Supplementary Materials, Figure S2).

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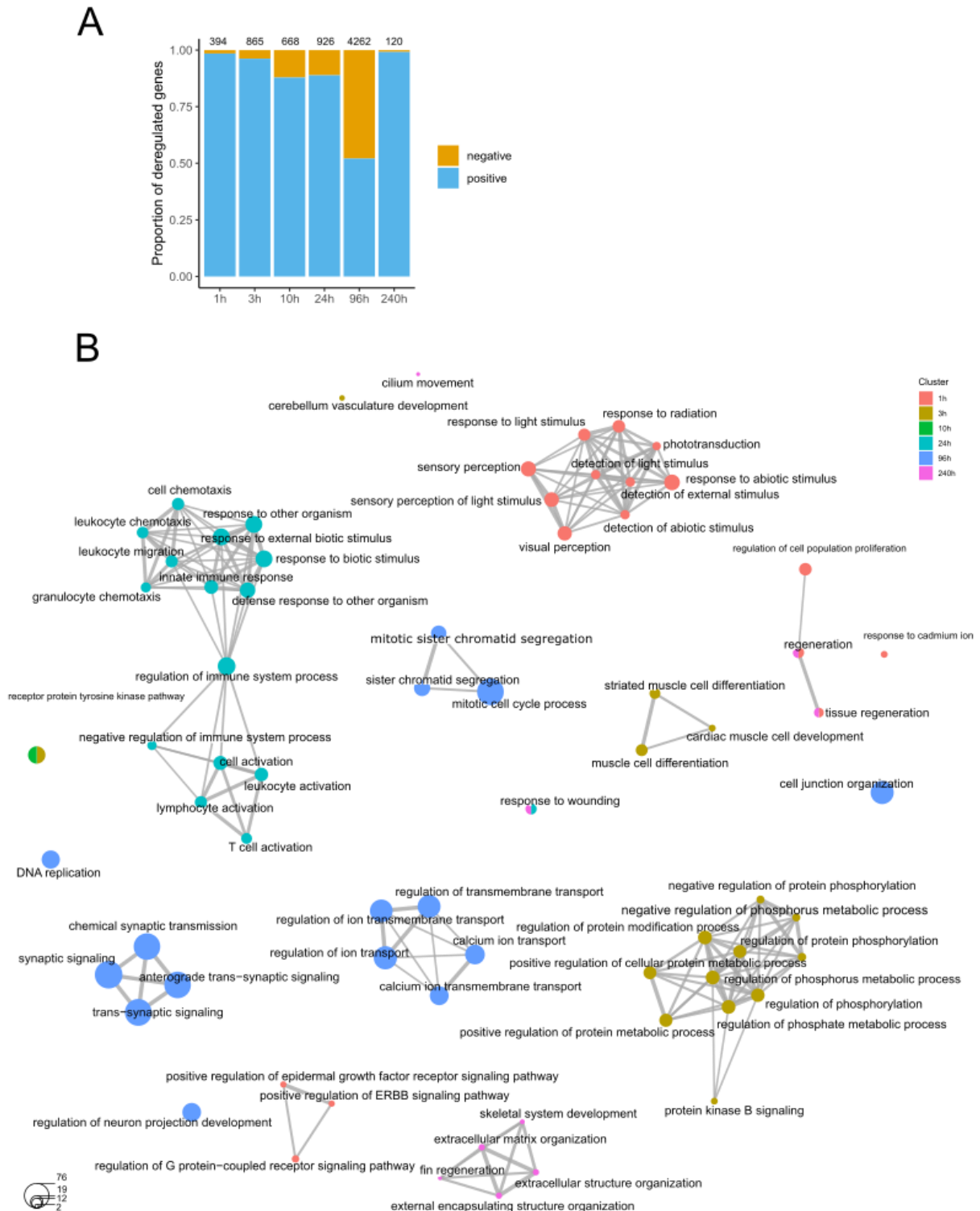


Figure 1: Differential expression analysis in injured telencephalon compared to uninjured control. (a) Proportion of dysregulated genes after stabwound injury at 1 hpl, 3 hpl, 10 hpl, 24 hpl, 96 hpl and 240 hpl. In orange are represented the negative regulated genes and in blue the positive regulated genes. On the top of the graph are specified the total number of DEG. **(b)** Over-representation analysis through Go terms on Biological Process.

To further assess the type of cells mobilized by regeneration at very early stages after injury, we characterized the expression pattern of several candidate genes upregulated at 1hpl. The activating transcription factor 3 (*atf3*), known to be involved in spinal cord regenerative neurogenesis and suggested to regulate inflammatory response (Wang et al., 2017), was expressed in cells located in the VZ of the dorsal and lateral telencephalon under control condition. Ectopic *atf3* expression was detected alongside the stabwound injury site (white arrow in the magnification picture). Moreover, comparing to the uninjured hemisphere, we could distinguish two ectopic areas of expression: one peripheral to the medial ventricular zone and another one near the dorsal proliferation zone (DPZ) (**Figure 2A**). We also checked for the expression of arylalkylamine N-acetyltransferase 2 (*aanat2*), known to be involved in melatonin synthesis and thus usually present in various cells of the pineal gland including photoreceptors. *Aanat2* positive cells were present in the dorso-medial ventricular zone of the dorsal telencephalon and in the parenchyma corresponding to the physical lesion made by the stab wound, visible as a blood clot (Kroehne et al., 2011) (**Figure 2B**). We then checked the expression of dopa-decarboxylase (*ddc*) involved in the synthesis of dopamine and serotonin, we observed cells along the injury site in the parenchyma (**Figure 2C**). These results confirm the upregulation of *atf3*, *aanat2* and *ddc* observed by RNAseq and suggest that injury led to the immediate early response at the transcriptome level as early as 1 hpl.

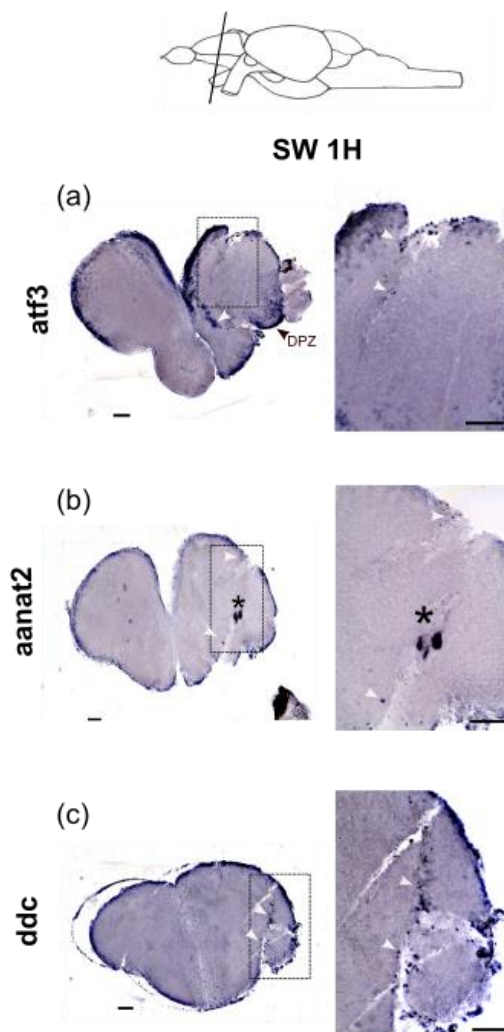


Figure 2: RNA *in situ* hybridization on adult brain sections showing expression of *atf3*, *aanat2* and *ddc* one hour post lesion. (a) Coronal sections of brain showing *atf3*. (b) Expression of *aanat2*. The black asterisk represents the blood clot. (c) Expression of *ddc*. The black rectangle represents the area for the higher magnification images. Scale bar = 100 μ m. The scheme at the top represents the location of the coronal sections in the brain.

4.2. Kinetics of relative gene expression in the injured telencephalon

We performed a kinetic analysis using relative gene expression levels in the injured telencephalon in order to characterize the dynamic of the response after injury over time. We first used hierarchical non supervised clustering and detected eight

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different groups with maximum of expression at different time post lesion (cluster 1 to 8 on **Figure 3A**). To better describe the kinetic of expression across time, we next applied hierarchical clustering on the mean gene expression of clusters obtained after fuzzy-mean clustering (**Figure 3B**). Six clusters (from A to F) were identified with maximum of expression at the different time post lesion. The enrichment of Gene Ontology (GO) analysis on each cluster characterized the pathways modulated over time after injury.

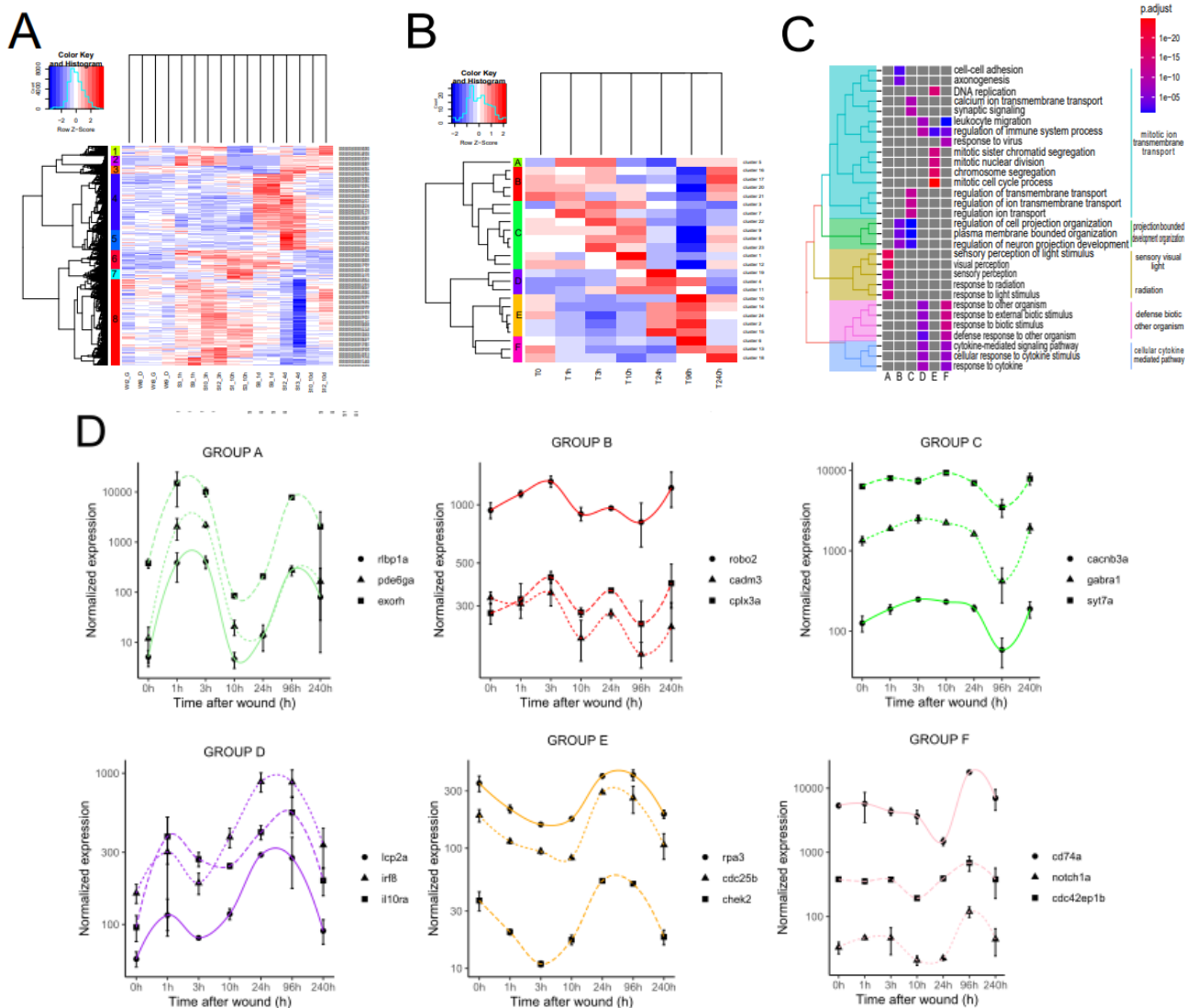


Figure 3: Kinetic analysis of relative gene expression in the injured telencephalon.

(a) Hierarchical non supervised clustering resulting in 8 clusters. **(b)** Fuzzy mean clustering obtained from the mean gene expression of clusters and resulting in 6 clusters (from A to F). **(c)** GO enrichment of biological processes showing the top enriched pathways from Group A to Group F. **(d)** Relative expression of gene candidates from the base expression level before wound (0 hpl) and up to 240 hpl.

Group A was composed of genes with biphasic expression: high levels of relative gene expression at 1hpl and 3hpl, decreased expression between 10 hpl and 24 hpl, followed by a second increase of expression levels at 96 hpl and onward (**Figure 3B**). This group included genes involved in modulation of the response to light perception and the response to radiation (**Figure 3C**), such as retinaldehyde binding protein 1a (*rlbp1a*) involved in optokinetic behavior, phosphodiesterase 6Ga (*pde6ga*) predicted to be involved in activation of MAPK activity and expressed in epiphysis, retinal photoreceptor layer and retinal rod cell and the gene extra-ocular rhodopsin (*exorh*) which plays a role in absorption of visible light and circadian regulation of gene expression (**Figure 3D**). **Group B** corresponded to genes with strong expression at 1 hpl and 3 hpl, lower expression at 96 hpl followed by a peak of expression at 240hpl (**Figure 3B**). Cell-cell adhesion, axonogenesis and regulation of cell projection organization were the main modulated pathways characterizing this group (**Figure 3C**). It included genes such as roundabout, axon guidance receptor, homolog 2 (*robo2*), cell adhesion molecule 3 (*cadm3*) and complexin 3a (*cplx3a*) involved respectively in axonal guidance, cell adhesion and regulation of neurotransmitter secretion (**Figure 3D**). **Group C** was characterized by the decrease of expression at 96 hpl and strong expressions at 3 hpl and 10 hpl (**Figure 3B, D**). Genes involved in the modulation of ion transport and synaptic signaling were enriched (**Figure 3C**), including the genes calcium channel, voltage-dependent beta 3a (*cacnb3a*), Gamma-aminobutyric acid (GABA) A receptor alpha 1 (*gabra1*) acting on GABAergic synaptic transmission and synaptotagmin VIIa (*syt7a*) which plays several functions as calcium-ion regulated exocytosis and regulation of secretion by cell (**Figure 3D**). **Group D** included high relative expression of genes from 24 hpl to 96 hpl, such as Lymphocyte cytosolic protein 2a (*lcp2a*), interferon regulatory factor 8 (*irf8*) and interleukin 10 receptor, alpha (*il10ra*) which are respectively involved in intracellular signal transduction and transmembrane receptor protein tyrosine kinase signaling pathway, regulation of macrophage differentiation, regulation of neutrophil differentiation and in cytokine-mediated signaling pathway (**Figure 3D**). Go term analysis confirmed the role of genes included in that group that mainly act on the regulation of the immune system (cellular cytokine mediated pathway, leukocyte migration) (**Figure 3C**). **Group E** and **Group F** could be identified as the late response groups with high levels of gene expression starting at 24 hpl to 96 hpl for group E and 96 hpl to 240 hpl for Group F (**Figure 3B**). Genes included in group E regulated mitotic nuclear division, DNA

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replication, chromosome segregation and mitotic cell cycle process and as in Group D, were involved in the regulation of the immune system. Example of expression kinetic for replication protein A3 (*rpa3*), cell division cycle 25B (*cdc25b*) and checkpoint kinase 2 (*chk2*) are provided (**Figure 3C**). **Group F** was described by high relative gene expression at 240 hpl. The main modulated pathways acted on the regulation of the immune system as illustrated through the relative expression of *cd74a* involved in T cell activation involved in immune response (**Figure 3B-C**). Two other genes included CDC42 effector protein (Rho GTPase binding) 1b (*cdc42ep1b*) acting on positive regulation of cellular component organization and regulation of cell shape and Notch receptor 1a (*notch1a*) that regulates cell fate specification (**Figure 3D**). Altogether, we described a succession of events representative of the biological processes occurring after stabwound injury from 1hpl to 240 hpl. The immediate early response is characterized by the upregulation of genes with function in visual perception, followed by the modulation of axonogenesis and cell adhesion. Afterwards, modifications on synaptic signaling and ion transport were observed followed by immune system response. This latter regulation was also modulated on all the following groups of clusters, but Group E was mainly described with modulation on cell cycle progression.

4.3. Comparing chronic low to moderate dose rates of ionizing radiation to stabwound injury

In order to compare the commonalities of effects on neurogenesis after injury and the response to IR, we compared the transcriptomics response during regeneration and after chronic exposure to 0.5 mGy.h⁻¹ and 5 mGy.h⁻¹ of gamma rays.

Hierarchical clustering of the transcriptomics data obtained after stabwound and chronic irradiation showed similarity with both datasets (**Figure 4A**). By checking the number of genes regulated in common between the two datasets, few genes were shared between the irradiated conditions and 10hpl and 24 hpl (**Figure 4A** and data not shown).

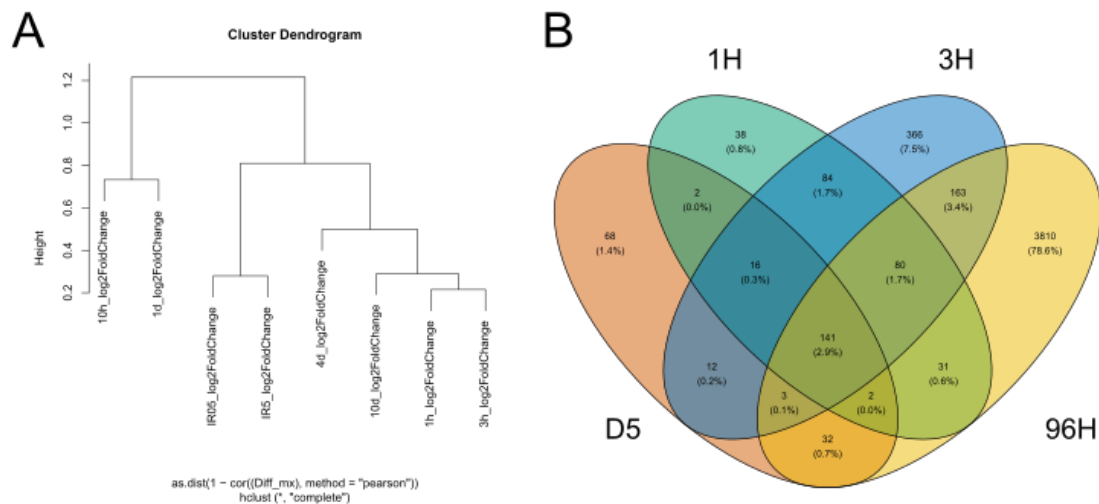


Figure 4: Overall responses after chronic exposure to low to moderate dose rates of ionizing radiation compared to stabwound injury. (a) Dendrogram from hierarchical clustering including both datasets. **(b)** Venn diagram of differentially expressed genes 1hpl, 3hpl, 96hpl and after 5 mGy.h⁻¹ irradiation ($|\text{fold change}| \geq 1.5$ and adjusted p-value < 0.05).

To further compare the transcriptomics response after irradiation and after injury we focused on D5 DEGs where many genes were dysregulated, and the response at 1 hpl, 3 hpl and 96 hpl. This way we found 161 DEGs, 172 DEGs and 178 DEGs in common with 1 hpl, 3 hpl and 96 hpl respectively (**Figure 4B**). Go term enrichment analysis demonstrated that these 4 sets of dysregulated genes contained genes involved in visual perception with genes such as *kerA*, *exorh* or *parapinopsinb* (GO:0007601, $\text{fdr} < 10^{-21}$), serotonin metabolism process with upregulation of *ddc*, *tph2* and *tph1a* (GO:0042428, $\text{fdr} = 0.0006$), retinoid binding (GO:0005501, 1 hpl: $\text{fdr} < 10^{-06}$, 3 hpl: $\text{fdr} < 10^{-06}$) (*cyp27C1*, *rbp5*, *exorh*, *rbp3*, *rbp4l*) and pineal gland development (GO:0021982, 1 hpl: $\text{fdr} = 0.0005$, 3 hpl: $\text{fdr} = 0.0006$) with upregulation of *noto*, *bsx* and *nr2e3*. (**Figure 5A and Supplementary materials, Table S1**). The analysis of the expression pattern of *aanat2* (for melatonin synthesis) in the adult brain zebrafish confirms the presence of ectopic *aanat2* cells in the parenchyma at 5 mGy.h⁻¹ irradiation, (**Figure 5B**).

Scientific Paper: Reactive neurogenesis is induced after chronic exposure to ionizing radiation at moderate dose rates in adult zebrafish

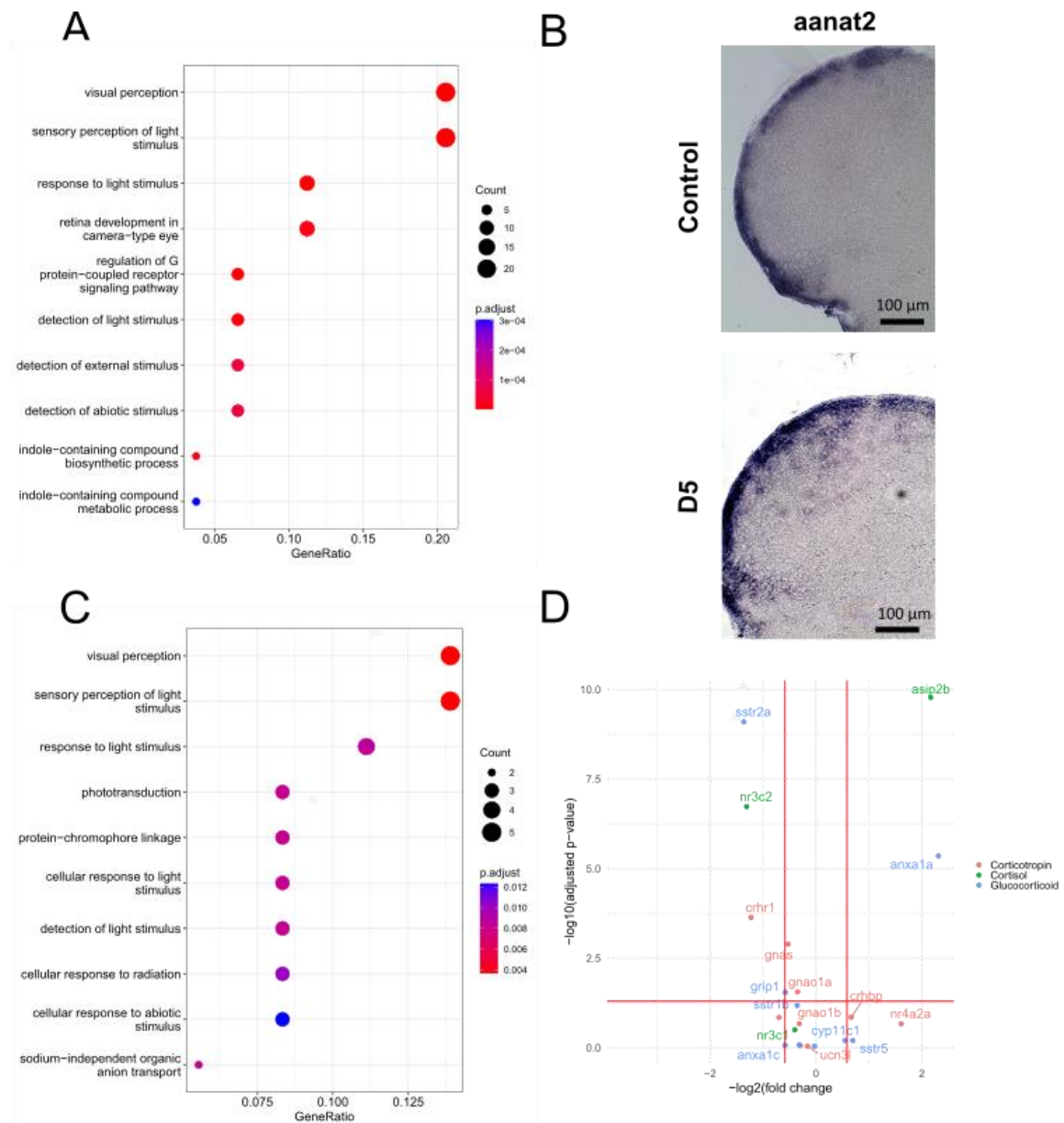


Figure 5: Transcriptomic effects in both chronic exposure to low to moderate dose rates of ionizing radiation and after stabwound injury. (a) GO enrichment of biological processes showing the top enriched pathways in common between 1 hpl, 3 hpl, 96 hpl and after 5 mGy.h⁻¹ irradiation. **(b)** RNA *in situ* hybridization on adult brain section showing expression of *aanat2* in control and 5 mGy.h⁻¹ irradiated fish. Scale bar = 100 μm. **(c)** GO enrichment of biological process unique to 5 mGy.h⁻¹ irradiated fish. **(d)** Volcano plot of differentially expressed genes related to cortisol, corticotropin and glucocorticoids at 96 hpl.

We then checked the potential pathways dysregulated specifically at 5 mGy.h⁻¹. 68 DEGs were unique to 5 mGy.h⁻¹ irradiated fish in comparison to the stages 1hpl, 3hpl and 96hpl. Go term analysis on these genes showed again enrichment in visual perception (GO:0007601, *fdr*=0.004) and phototransduction (GO:0007602, *fdr*=0.009) with genes specific to that condition such as *crx*, *parapinopsina*, *opn1lw1*, *opn1lw2*, *rcvrn2* (**Figure 5C**). Moreover, several genes involved in stimulation of cortisol secretion (*cyp11c1*, *nr4a1*) were upregulated and specific to the irradiated condition while *mrap2a* was downregulated. As cortisol is known to regulate inflammation, we checked if the inflammatory response observed after stab wound was also detected in irradiated fish. At 1 hpl, 3 hpl and 10 hpl the upregulation of *nr4a3* was observed (data not shown). Other genes involved in ACTH or cortisol releases (*crhr1*, *mrap2b*, *sstr2a*) were downregulated at 96 hpl while *hsd11b1la*, *anxa1a* and *anxa3b* were upregulated (**Figure 5D**). None of these were modulated in D5 (data not shown), with the exception of *nr3c2* upregulated at D5 and downregulated at 96hpl (**Figure 5D and data not shown**).

5. Discussion

In this study, we used adult zebrafish to better characterize the molecular effects on neurogenesis induced either by physical injury, known to be dependent on neuroinflammation, or as a result of the stress response induced by IR. We employed transcriptomic analyses on the telencephalon and assessed gene expression of candidate genes using RNA *in situ* hybridization on adult brain sections. This strategy allowed us to assess immediate early responses after both stabwound injury and chronic exposure to low and moderate dose rates of IR.

We ensured the validity of the transcriptomics study of the injury response after wounding by measuring the expression levels of *pcna*, *bdnf*, *cxcr4b*, *socs3*, and *id1*, that are known to be highly upregulated in the lesioned hemisphere (Cacialli et al., 2018; Cameron et al., 2005; Hui et al., 2014; Viales et al., 2015). In addition, apoptosis was proposed to play a major role to resolve inflammation during tissue repair (Brown et al., 1997; Wu and Chen, 2014) and was described to be stimulated as early as 20 hpl by RNA sequencing and 1 dpl by RT-qPCR in zebrafish telencephalon (Anand et al., 2021; Demirci et al., 2020). Here, we observed activation of apoptosis at a later time after injury, at 96hpl (*tradd*, *casp8*, *casp3b*, *fas*, *bida*), which could indicate differences in the data analysis (depth of

sequencing) or modalities in performing the wounding. Activation of the immune system has been well described during the process of regenerative neurogenesis (Caldwell et al., 2019; Cavone et al., 2021; Kizil et al., 2012a). Indeed, this process is involved in the regulation of the acute inflammatory response for proper regeneration and is mandatory since microglia has been demonstrated to eliminate dead cells after brain injury in zebrafish (Harry and Kraft, 2012; Herzog et al., 2019). Regarding our results, the activated immune response was detected at 24 hpl and 96 hpl through various pathways such as cytokine-mediated pathway, lymphocyte activation or innate immune response, which is in accordance with previous studies showing that recruitment of microglia peaks at 24 hpl up to 96 hpl (Kanagaraj et al., 2020). Furthermore, we detected the activation of DNA replication at 96 hpl that marks the beginning of the proliferation stage which is in accordance with other studies (Demirci et al., 2020; Shimizu et al., 2021). This step is held by ventricular radial glial that function as neuronal progenitors to give rise to neuroblasts that will finally terminate into the parenchyma and differentiate into neurons to replace lost neurons to the injury site (Kroehne et al., 2011). Herein, we could here validate our model of wounding the brain of zebrafish through transcriptomics analysis.

We aimed at analyzing our data from stabwound injury through over-representation analysis (ORA) and kinetics of relative gene expression analysis. The results were consistent and pointed out on several regulated processes such as visual perception, axonogenesis, ion transport and even regulation of immune system and cell proliferation. Indeed, our data revealed the involvement of the immune system as soon as 1 hpl with enrichment of leukocyte migration through upregulation of genes as *myd88*, *il1b*, *cxcl18b* or *mmp9* and cell proliferation was negatively regulated as early as 1 hpl (GO:0008285; *tob1a*, *igfbp1a*, *mmp9*, *si:ch211-241b2.5*, *sh3bp4a*, *btg2*, *si:dkey-79d12.5*). The enrichment analysis revealed the modulation of genes mainly involved in calcium transport which is in agreement with precedent studies that described similar process during brain (Demirci et al., 2020) and spinal cord regeneration (Fu et al., 2019; Hui et al., 2014). Interestingly, we detected the upregulation of genes involved in ion transport with function in chloride, sodium-potassium and calcium channels at much earlier stage after injury (from 1 hpl onward). Bioelectric mechanisms are known to influence cell differentiation and proliferation, to direct mature neurons to re-enter the cell cycle, to be involved in apoptotic process or to be essential in neuroinflammation, notably for microglia activation (Cone Jr and Cone, 1976; Lauritzen et al., 1997, 2003; Levin, 2009;

Luo et al., 2021; Nuckels et al., 2009; Stillwell et al., 1973). Interestingly, we also could detect in a previous study with chronic exposure to low to moderate dose rates of IR (Cantabella et al., 2022) the enrichment in regulation of metal ion transmembrane transporter activity and anion transmembrane transporter activity including calcium or sodium transporters. However, the exact involvement and function of this transport in regenerative neurogenesis of zebrafish telencephalon as early as 1 hpl was not elucidated here and remain to be investigated.

Successful axonal regeneration is a key to restore CNS function after injury and is linked to acute inflammation (Becker and Becker, 2007; Hui et al., 2014). The stimulated pathways involve various axonal guidance molecules with functions in attraction, repulsion or adhesion necessary for axonal regrowth that are mobilized during normal brain development (Tessier-Lavigne and Goodman, 1996) and during regeneration (Becker et al., 2004). We observed at 96 hpl the dysregulation of *slit1a*, *sema6ba*, *sema6bb*, *sema6e*, *sema4ba*, *plxna1a* that have repellent properties and the ephrin *epha4b* that is involved in organization of axons during CNS development (Cameron et al., 2005; Flanagan, 2006; Hui et al., 2014; Xu et al., 1996). Additionally, *atf3* known to be upregulated in spinal cord and optic nerve regeneration including an action on axon regeneration, was overexpressed 1 hpl indicating an involvement in the immediate early response. We could also detect in previous work the regulation of axon extension involved in axon guidance when fish were irradiated at 0.5 mGy.h⁻¹ and 5 mGy.h⁻¹ continuously during 36 days suggesting the reactive neurogenesis after chronic stress caused by IR (Cantabella et al., 2022).

For the first time to our knowledge, we performed the comparison between uninjured and injured telencephalon at very early stages post lesion (1 hpl and 3 hpl). These times post lesion turned out to include genes involved in visual perception, response to light stimulus, phototransduction or retinoid binding which are pathways that were also dysregulated after IR exposure. Retinoids play a major role in vision since retinal can bind to opsin proteins and serve as chromophore of photopigment. Additionally, retinoic acid regulates opsin expression in developing photoreceptors and ultimately activates the phototransduction cascade (Cvekl and Wang, 2009; Fernández and Gisbert, 2011; Lamb and Pugh Jr, 2004; Palczewski, 2006; Prabhudesai et al., 2005) which is necessary to mediate the conversion of light photons into electrochemical signals (Zhao and Fent, 2016). We could also observe the enrichment of genes involved in pineal

gland development and the upregulation of *tph1a*, *tph2*, *aanat2* and *asmt* involved in melatonin synthesis in both stabwound and irradiated brains. RNA *in situ* hybridization data confirmed that *aanat2*, usually expressed in the pineal gland including in photoreceptors was also expressed in the telencephalon with localization in the parenchyma. Fernandes and colleagues shed light to the presence of photoreceptors in the preoptic area independent from eyes and pineal gland and with function in phototaxis. Consequently, our data suggest the existence of cells with photoreceptor role in the telencephalon through expression of *aanat2* and enrichment of visual perception. Similarly, in 48 hpf old larvae continuously exposed to IR at 5 mGy.h⁻¹ and 50 mGy.h⁻¹, the enrichment in phototransduction with genes as *rhodopsin*, *pde6g* and *s-antigen visual arrestin* and in visual system development including the genes *crx*, *rbp4l* and *rpgr1p1* were previously depicted (Murat El Houdigui et al., 2019). Overall, our data suggest that brain injury and induction of stress can activate the expression in the telencephalon of genes with function in phototransduction, but their exact role in regenerative neurogenesis remains unknown.

Cortisol secretion is regulated after both stabwound injury and exposure to moderate dose rates of IR with one shared gene and several genes specific to a given condition. Cortisol, as being part of glucocorticoids family, is well known for its anti-inflammatory role as illustrated through the suppression of the immune response after traumatic brain injury in mice (Dong et al., 2016) and could thus explain the absence of inflammatory markers in fish exposed to chronic low to moderate dose rates of IR by compensating this response. Several studies in either human hippocampal progenitor cell line, rhesus monkeys or rats have also demonstrated the inhibitory role of cortisol on neurogenesis (Anacker et al., 2013a, 2013b; Coe et al., 2003; Wong and Herbert, 2006). However, in some cases such as when acute stress is applied by 3h of immobilization in rats, the elevated plasma cortisol levels was proposed as an inducer of cell proliferation in the dentate gyrus of the hippocampus (Saaltink and Vreugdenhil, 2014). In fish, expression in the brain of *pcna*, *neurod1* and cortisol receptor genes were positively associated suggesting a positive effect of cortisol level on neurogenesis (Sadoul et al., 2018). Our data on chronic exposure to low and moderate dose rates of IR do not report the induction of neuroinflammation however we cannot exclude that inflammation was induced during the initial phase of IR exposure and was subsequently repressed. More data are needed in order to assess the interplay between inflammation, stress and

neurogenesis, especially in the context of chronic exposure to IR where compensatory and acclimation mechanisms can modify the physiological response over time.

6. Conclusion

In this study, we aimed at deciphering the molecular effects of IR on adult neurogenesis and the potential involvement of neuroinflammation under chronic stress by comparing the response to brain regeneration after stabwound injury. Our results demonstrate the fine regulation of ion transporters, axonogenesis and the upregulation of genes involved in visual perception very early (1 hpl) after stabwound and chronic exposure to low to moderate dose rate of IR revealing their involvement for proper regeneration. We highlight the presence of cells expressing *aanat2* in the parenchyma of the telencephalon which also suggest the presence of cells expressing genes with photoreceptor role in this brain area that can be targeted by IR. Since genes with function in inflammation were not dysregulated after IR, we propose that cortisol may play a role in controlling inflammation under chronic stress. Altogether, since our data revealed several common regulated pathways after stabwound injury and chronic stress, we strongly suggest the stimulation of reactive neurogenesis after chronic exposure to low to moderate dose rates of ionizing radiation with an unanticipated role of genes with function in visual perception in the immediate early response to stress and injury.

7. References

- Adolf, B., Chapouton, P., Lam, C.S., Topp, S., Tannhäuser, B., Strähle, U., Götz, M., Bally-Cuif, L., 2006. Conserved and acquired features of adult neurogenesis in the zebrafish telencephalon. *Dev. Biol.* 295, 278–293.
- Alunni, A., Bally-Cuif, L., 2016. A comparative view of regenerative neurogenesis in vertebrates. *Development* 143, 741–753.
- Anacker, C., Cattaneo, A., Luoni, A., Musaelyan, K., Zunszain, P.A., Milanese, E., Rybka, J., Berry, A., Cirulli, F., Thuret, S., 2013a. Glucocorticoid-related molecular signaling pathways regulating hippocampal neurogenesis. *Neuropsychopharmacology* 38, 872–883.
- Anacker, C., Cattaneo, A., Musaelyan, K., Zunszain, P.A., Horowitz, M., Molteni, R., Luoni, A., Calabrese, F., Tansey, K., Gennarelli, M., 2013b. Role for the kinase SGK1 in stress, depression, and glucocorticoid effects on hippocampal neurogenesis. *Proc. Natl. Acad. Sci.* 110, 8708–8713.

- Anand, S.K., Sahu, M.R., Mondal, A.C., 2021. Induction of oxidative stress and apoptosis in the injured brain: potential relevance to brain regeneration in zebrafish. *Mol. Biol. Rep.* 48, 5099–5108.
- Armant, O., Gombeau, K., Murat El Houdigui, S., Floriani, M., Camilleri, V., Cavalie, I., Adam-Guillermin, C., 2017. Zebrafish exposure to environmentally relevant concentration of depleted uranium impairs progeny development at the molecular and histological levels. *PLoS One* 12, e0177932.
- Armant, O., März, M., Schmidt, R., Ferg, M., Diotel, N., Ertzer, R., Bryne, J.C., Yang, L., Baader, I., Reischl, M., 2013. Genome-wide, whole mount in situ analysis of transcriptional regulators in zebrafish embryos. *Dev. Biol.* 380, 351–362.
- Azizova, T.V., Bannikova, M.V., Grigoryeva, E.S., Rybkina, V.L., Hamada, N., 2020. Occupational exposure to chronic ionizing radiation increases risk of Parkinson's disease incidence in Russian Mayak workers. *Int. J. Epidemiol.* 49, 435–447. <https://doi.org/10.1093/ije/dyz230>
- Becker, C.G., Becker, T., 2007. Growth and pathfinding of regenerating axons in the optic projection of adult fish. *J. Neurosci. Res.* 85, 2793–2799.
- Becker, C.G., Schweitzer, J., Feldner, J., Schachner, M., Becker, T., 2004. Tenascin-R as a repellent guidance molecule for newly growing and regenerating optic axons in adult zebrafish. *Mol. Cell. Neurosci.* 26, 376–389.
- Becker, T., Wullmann, M.F., Becker, C.G., Bernhardt, R.R., Schachner, M., 1997. Axonal regrowth after spinal cord transection in adult zebrafish. *J. Comp. Neurol.* 377, 577–595.
- Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B Methodol.* 57, 289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>
- Brown, D.L., Kao, W.W., Greenhalgh, D.G., 1997. Apoptosis down-regulates inflammation under the advancing epithelial wound edge: delayed patterns in diabetes and improvement with topical growth factors. *Surgery* 121, 372–380.
- Cacialli, P., D'angelo, L., Kah, O., Coumailleau, P., Gueguen, M., Pellegrini, E., Lucini, C., 2018. Neuronal expression of brain derived neurotrophic factor in the injured telencephalon of adult zebrafish. *J. Comp. Neurol.* 526, 569–582.
- Caldwell, L.J., Davies, N.O., Cavone, L., Mysiak, K.S., Semenova, S.A., Panula, P., Armstrong, J.D., Becker, C.G., Becker, T., 2019. Regeneration of Dopaminergic Neurons in Adult Zebrafish Depends on Immune System Activation and Differs for Distinct Populations. *J. Neurosci.* 39, 4694. <https://doi.org/10.1523/JNEUROSCI.2706-18.2019>
- Cameron, D.A., Gentile, K.L., Middleton, F.A., Yurco, P., 2005. Gene expression profiles of intact and regenerating zebrafish retina. *Mol Vis* 11, 91.
- Cantabella, E., Camilleri, V., Cavalie, I., Dubourg, N., Gagnaire, B., Charlier, T.D., Adam-Guillermin, C., Cousin, X., Armant, O., 2022. Revealing the Increased Stress Response Behavior through Transcriptomic Analysis of Adult Zebrafish Brain after Chronic Low to Moderate Dose Rates of Ionizing Radiation. *Cancers* 14, 3793.
- Cavone, L., McCann, T., Drake, L.K., Aguzzi, E.A., Opreșoreanu, A.-M., Pedersen, E., Sandi, S., Selvarajah, J., Tsarouchas, T.M., Wehner, D., 2021. A unique macrophage subpopulation signals directly to progenitor cells to promote regenerative neurogenesis in the zebrafish spinal cord. *Dev. Cell* 56, 1617–1630.
- Coe, C.L., Kramer, M., Czéh, B., Gould, E., Reeves, A.J., Kirschbaum, C., Fuchs, E., 2003. Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile rhesus monkeys. *Biol. Psychiatry* 54, 1025–1034.

Scientific Paper: Reactive neurogenesis is induced after chronic exposure to ionizing radiation at moderate dose rates in adult zebrafish

- Cone Jr, C.D., Cone, C.M., 1976. Induction of mitosis in mature neurons in central nervous system by sustained depolarization. *Science* 192, 155–158.
- Curtis, M.A., Kam, M., Nannmark, U., Anderson, M.F., Axell, M.Z., Wikkelso, C., Holtås, S., van Roon-Mom, W.M., Bjork-Eriksson, T., Nordborg, C., 2007. Human neuroblastsmigrate to the olfactory bulb via a lateral ventricular extension. *science* 315, 1243–1249.
- Cvekl, A., Wang, W.-L., 2009. Retinoic acid signaling in mammalian eye development. *Exp. Eye Res.* 89, 280–291.
- Demirci, Y., Cucun, G., Poyraz, Y.K., Mohammed, S., Heger, G., Papatheodorou, I., Ozhan, G., 2020. Comparative transcriptome analysis of the regenerating zebrafish telencephalon unravels a resource with key pathways during two early stages and activation of wnt/ β -catenin signaling at the early wound healing stage. *Front. Cell Dev. Biol.* 8, 584604.
- Diotel, N., Lübke, L., Strähle, U., Rastegar, S., 2020. Common and distinct features of adult neurogenesis and regeneration in the telencephalon of zebrafish and mammals. *Front. Neurosci.* 957.
- Dong, T., Zhi, L., Bhayana, B., Wu, M.X., 2016. Cortisol-induced immune suppression by a blockade of lymphocyte egress in traumatic brain injury. *J. Neuroinflammation* 13, 1–13.
- Dunn, K., Yoshimaru, H., Otake, M., ANNEGERS, J.F., Schull, W.J., 1990. Prenatal exposure to ionizing radiation and subsequent development of seizures. *Am. J. Epidemiol.* 131, 114–123.
- Durinck, S., Spellman, P.T., Birney, E., Huber, W., 2009. Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. *Nat. Protoc.* 4, 1184–1191.
- Farin, A., Liu, C.Y., Langmoen, I.A., Apuzzo, M.L., 2009. The biological restoration of central nervous system architecture and function: part 2—emergence of the realization of adult neurogenesis. *Neurosurgery* 64, 581–601.
- Fernández, I., Gisbert, E., 2011. The effect of vitamin A on flatfish development and skeletogenesis: a review. *Aquaculture* 315, 34–48.
- Flanagan, J.G., 2006. Neural map specification by gradients. *Curr. Opin. Neurobiol.* 16, 59–66.
- Folley, J.H., Borges, W., Yamawaki, T., 1952. Incidence of leukemia in survivors of the atomic bomb in Hiroshima and Nagasaki, Japan. *Am. J. Med.* 13, 311–321.
- Fu, H., Han, G., Li, H., Liang, X., Hu, D., Zhang, L., Tang, P., 2019. Identification of key genes and pathways involved in the heterogeneity of intrinsic growth ability between neurons after spinal cord injury in adult zebrafish. *Neurochem. Res.* 44, 2057–2067.
- Gillies, M., Richardson, D.B., Cardis, E., Daniels, R.D., O'Hagan, J.A., Haylock, R., Laurier, D., Leuraud, K., Moissonnier, M., Schubauer-Berigan, M.K., 2017. Mortality from circulatory diseases and other non-cancer outcomes among nuclear workers in France, the United Kingdom and the United States (INWORKS). *Radiat. Res.* 188, 276–290.
- Grandel, H., Kaslin, J., Ganz, J., Wenzel, I., Brand, M., 2006. Neural stem cells and neurogenesis in the adult zebrafish brain: origin, proliferation dynamics, migration and cell fate. *Dev. Biol.* 295, 263–277.
- Harry, G.J., Kraft, A.D., 2012. Microglia in the developing brain: a potential target with lifetime effects. *Neurotoxicology* 33, 191–206.

- Hellström, N.A., Björk-Eriksson, T., Blomgren, K., Kuhn, H.G., 2009. Differential recovery of neural stem cells in the subventricular zone and dentate gyrus after ionizing radiation. *Stem Cells* 27, 634–641.
- Herzog, C., Pons Garcia, L., Keatinge, M., Greenald, D., Moritz, C., Peri, F., Herrgen, L., 2019. Rapid clearance of cellular debris by microglia limits secondary neuronal cell death after brain injury in vivo. *Development* 146, dev174698.
- Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., Muffato, M., Collins, J.E., Humphray, S., McLaren, K., Matthews, L., 2013. The zebrafish reference genome sequence and its relationship to the human genome. *Nature* 496, 498–503.
- Hui, S.P., Sengupta, D., Lee, S.G.P., Sen, T., Kundu, S., Mathavan, S., Ghosh, S., 2014. Genome wide expression profiling during spinal cord regeneration identifies comprehensive cellular responses in zebrafish. *PloS One* 9, e84212.
- Kalueff, A.V., Stewart, A.M., Gerlai, R., 2014. Zebrafish as an emerging model for studying complex brain disorders. *Trends Pharmacol. Sci.* 35, 63–75.
- Kanagaraj, P., Chen, J.Y., Skaggs, K., Qadeer, Y., Conner, M., Cutler, N., Richmond, J., Kommidi, V., Poles, A., Affrunti, D., 2020. Microglia stimulate zebrafish brain repair via a specific inflammatory cascade. *BioRxiv*.
- Kizil, C., Dudczig, S., Kyritsis, N., Machate, A., Blaesche, J., Kroehne, V., Brand, M., 2012. The chemokine receptor *cxcr5* regulates the regenerative neurogenesis response in the adult zebrafish brain. *Neural Develop.* 7, 27. <https://doi.org/10.1186/1749-8104-7-27>
- Kroehne, V., Freudenreich, D., Hans, S., Kaslin, J., Brand, M., 2011. Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors. *Development* 138, 4831–4841.
- Kyritsis, N., Kizil, C., Zocher, S., Kroehne, V., Kaslin, J., Freudenreich, D., Iltzsche, A., Brand, M., 2012. Acute inflammation initiates the regenerative response in the adult zebrafish brain. *Science* 338, 1353–1356.
- Lamb, T., Pugh Jr, E.N., 2004. Dark adaptation and the retinoid cycle of vision. *Prog. Retin. Eye Res.* 23, 307–380.
- Lauritzen, I., De Weille, J.R., Lazdunski, M., 1997. The potassium channel opener (-)-cromakalim prevents glutamate-induced cell death in hippocampal neurons. *J. Neurochem.* 69, 1570–1579.
- Lauritzen, I., Zanzouri, M., Honoré, E., Duprat, F., Ehrengruber, M.U., Lazdunski, M., Patel, A.J., 2003. K⁺-dependent cerebellar granule neuron apoptosis: role of TASK leak K⁺ channels. *J. Biol. Chem.* 278, 32068–32076.
- Lazarini, F., Mouthon, M.-A., Gheusi, G., de Chaumont, F., Olivo-Marin, J.-C., Lamarque, S., Abrous, D.N., Boussin, F.D., Lledo, P.-M., 2009. Cellular and behavioral effects of cranial irradiation of the subventricular zone in adult mice. *PloS One* 4, e7017–e7017. <https://doi.org/10.1371/journal.pone.0007017>
- Levin, M., 2009. Bioelectric mechanisms in regeneration: unique aspects and future perspectives. Presented at the Seminars in cell & developmental biology, Elsevier, pp. 543–556.
- Luo, L., Song, S., Ezenwukwa, C.C., Jalali, S., Sun, B., Sun, D., 2021. Ion channels and transporters in microglial function in physiology and brain diseases. *Neurochem. Int.* 142, 104925.
- März, M., Schmidt, R., Rastegar, S., Strähle, U., 2011. Regenerative response following stab injury in the adult zebrafish telencephalon. *Dev. Dyn.* 240, 2221–2231.
- Moe, M.C., Varghese, M., Danilov, A.I., Westerlund, U., Ramm-Petersen, J., Brundin, L., Svensson, M., Berg-Johnsen, J., Langmoen, I.A., 2005. Multipotent progenitor cells

- from the adult human brain: neurophysiological differentiation to mature neurons. *Brain* 128, 2189–2199.
- Murat El Houdigui, S., Adam-Guillermin, C., Armant, O., 2020. Ionising Radiation Induces Promoter DNA Hypomethylation and Perturbs Transcriptional Activity of Genes Involved in Morphogenesis during Gastrulation in Zebrafish. *Int. J. Mol. Sci.* 21, 4014.
- Murat El Houdigui, S., Adam-Guillermin, C., Loro, G., Arcanjo, C., Frelon, S., Floriani, M., Dubourg, N., Baudelet, E., Audebert, S., Camoin, L., 2019. A systems biology approach reveals neuronal and muscle developmental defects after chronic exposure to ionising radiation in zebrafish. *Sci. Rep.* 9, 1–15.
- Nuckels, R.J., Ng, A., Darland, T., Gross, J.M., 2009. The vacuolar-ATPase complex regulates retinoblast proliferation and survival, photoreceptor morphogenesis, and pigmentation in the zebrafish eye. *Invest. Ophthalmol. Vis. Sci.* 50, 893–905.
- Otake, M., Schull, W.J., 1998. Radiation-related brain damage and growth retardation among the prenatally exposed atomic bomb survivors. *Int. J. Radiat. Biol.* 74, 159–171.
- Palczewski, K., 2006. G protein-coupled receptor rhodopsin. *Annu Rev Biochem* 75, 743–767.
- Plummer, G., 1952. Anomalies occurring in children exposed in utero to the atomic bomb in Hiroshima. *Pediatrics* 10, 687–693.
- Prabhudesai, S.N., Cameron, D.A., Stenkamp, D.L., 2005. Targeted effects of retinoic acid signaling upon photoreceptor development in zebrafish. *Dev. Biol.* 287, 157–167.
- Raymond, P.A., Barthel, L.K., Bernardos, R.L., Perkowski, J.J., 2006. Molecular characterization of retinal stem cells and their niches in adult zebrafish. *BMC Dev. Biol.* 6, 1–17.
- Reynolds, B.A., Weiss, S., 1992. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *science* 255, 1707–1710.
- Saaltink, D.-J., Vreugdenhil, E., 2014. Stress, glucocorticoid receptors, and adult neurogenesis: a balance between excitation and inhibition? *Cell. Mol. Life Sci.* 71, 2499–2515.
- Sadoul, B., Alfonso, S., Bessa, E., Bouchareb, A., Blondeau-Bidet, E., Clair, P., Chatain, B., Begout, M.-L., Geffroy, B., 2018. Enhanced brain expression of genes related to cell proliferation and neural differentiation is associated with cortisol receptor expression in fishes. *Gen. Comp. Endocrinol.* 267, 76–81.
- Sanai, N., Nguyen, T., Ihrie, R.A., Mirzadeh, Z., Tsai, H.-H., Wong, M., Gupta, N., Berger, M.S., Huang, E., Garcia-Verdugo, J.-M., 2011. Corridors of migrating neurons in the human brain and their decline during infancy. *Nature* 478, 382–386.
- Schmidt, R., Beil, T., Strähle, U., Rastegar, S., 2014. Stab wound injury of the zebrafish adult telencephalon: a method to investigate vertebrate brain neurogenesis and regeneration. *JoVE J. Vis. Exp.* e51753.
- Schmidt, R., Strähle, U., Scholpp, S., 2013. Neurogenesis in zebrafish – from embryo to adult. *Neural Develop.* 8, 3. <https://doi.org/10.1186/1749-8104-8-3>
- Schull, W.J., Otake, M., 1999. Cognitive function and prenatal exposure to ionizing radiation. *Teratology* 59, 222–226.
- Shimizu, Y., Kiyooka, M., Ohshima, T., 2021. Transcriptome analyses reveal IL6/Stat3 signaling involvement in radial glia proliferation after stab wound injury in the adult Zebrafish optic tectum. *Front. Cell Dev. Biol.* 9, 668408.
- Stillwell, E., Cone, CM, Cone, CD, 1973. Stimulation of DNA synthesis in CNS neurones by sustained depolarisation. *Nature. New Biol.* 246, 110–111.

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- Tessier-Lavigne, M., Goodman, C.S., 1996. The molecular biology of axon guidance. *Science* 274, 1123–1133.
- Viales, R.R., Diotel, N., Ferg, M., Armant, O., Eich, J., Alunni, A., März, M., Bally-Cuif, L., Rastegar, S., Strähle, U., 2015. The helix-loop-helix protein id1 controls stem cell proliferation during regenerative neurogenesis in the adult zebrafish telencephalon. *Stem Cells* 33, 892–903.
- Wang, C., Liu, F., Liu, Y.-Y., Zhao, C.-H., You, Y., Wang, L., Zhang, J., Wei, B., Ma, T., Zhang, Q., 2011. Identification and characterization of neuroblasts in the subventricular zone and rostral migratory stream of the adult human brain. *Cell Res.* 21, 1534–1550.
- Wang, L.-F., Huang, S.-B., Zhao, H.-D., Liu, C.-J., Yao, L., Shen, Y.-Q., 2017. Activating transcription factor 3 promotes spinal cord regeneration of adult zebrafish. *Biochem. Biophys. Res. Commun.* 488, 522–527.
- Wong, E., Herbert, J., 2006. Raised circulating corticosterone inhibits neuronal differentiation of progenitor cells in the adult hippocampus. *Neuroscience* 137, 83–92.
- Wu, Y.S., Chen, S.N., 2014. Apoptotic cell: linkage of inflammation and wound healing.
- Xu, Q., Alldus, G., Macdonald, R., Wilkinson, D.G., Holder, N., 1996. Function of the Eph-related kinase rtk1 in patterning of the zebrafish forebrain. *Nature* 381, 319–322.
- Zhao, Y., Fent, K., 2016. Progestins alter photo-transduction cascade and circadian rhythm network in eyes of zebrafish (*Danio rerio*). *Sci. Rep.* 6, 1–10.

8. Supplementary materials

Figure S1 : Goterms enrichment of upregulated genes after stabwound injury

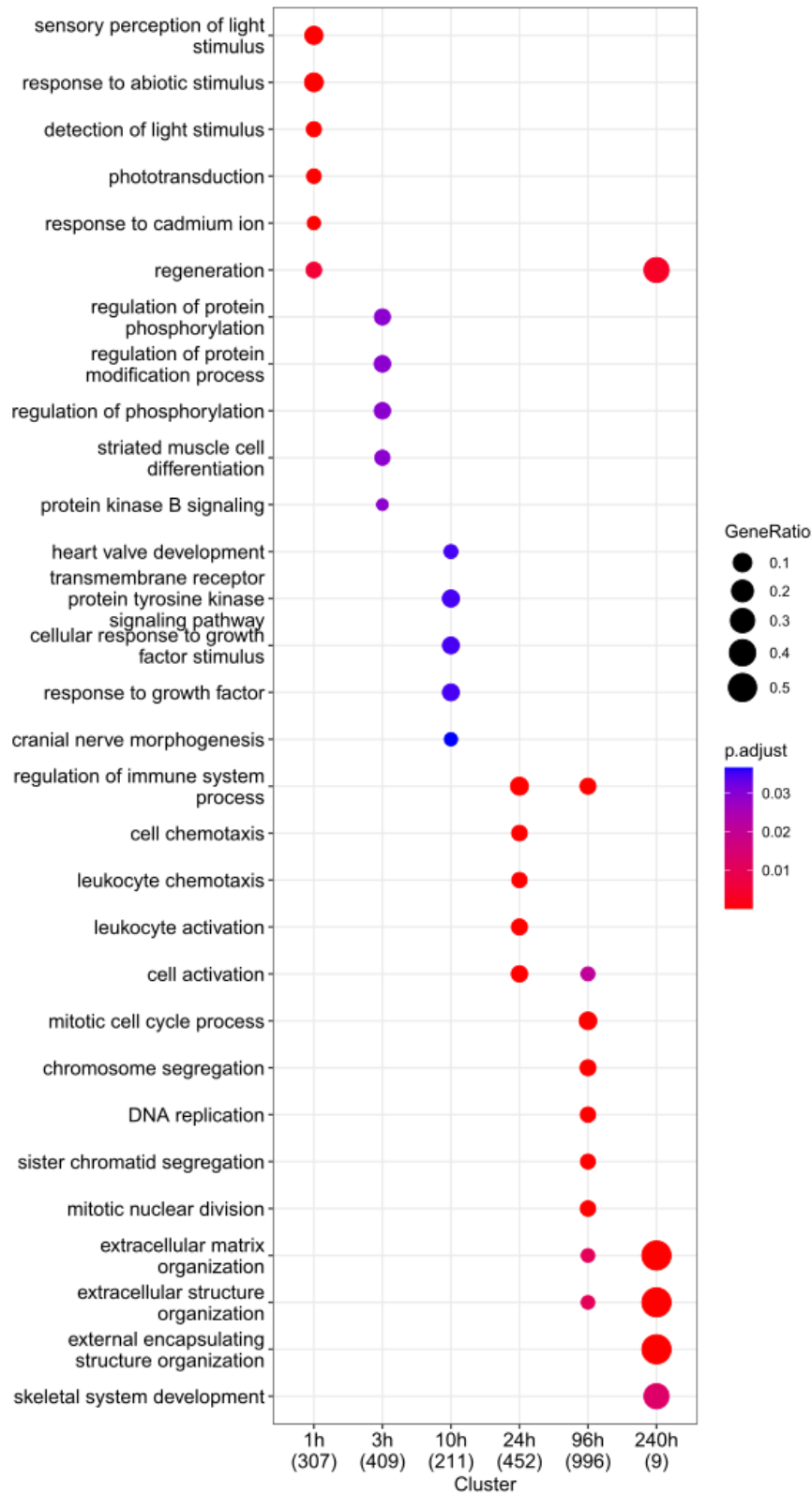
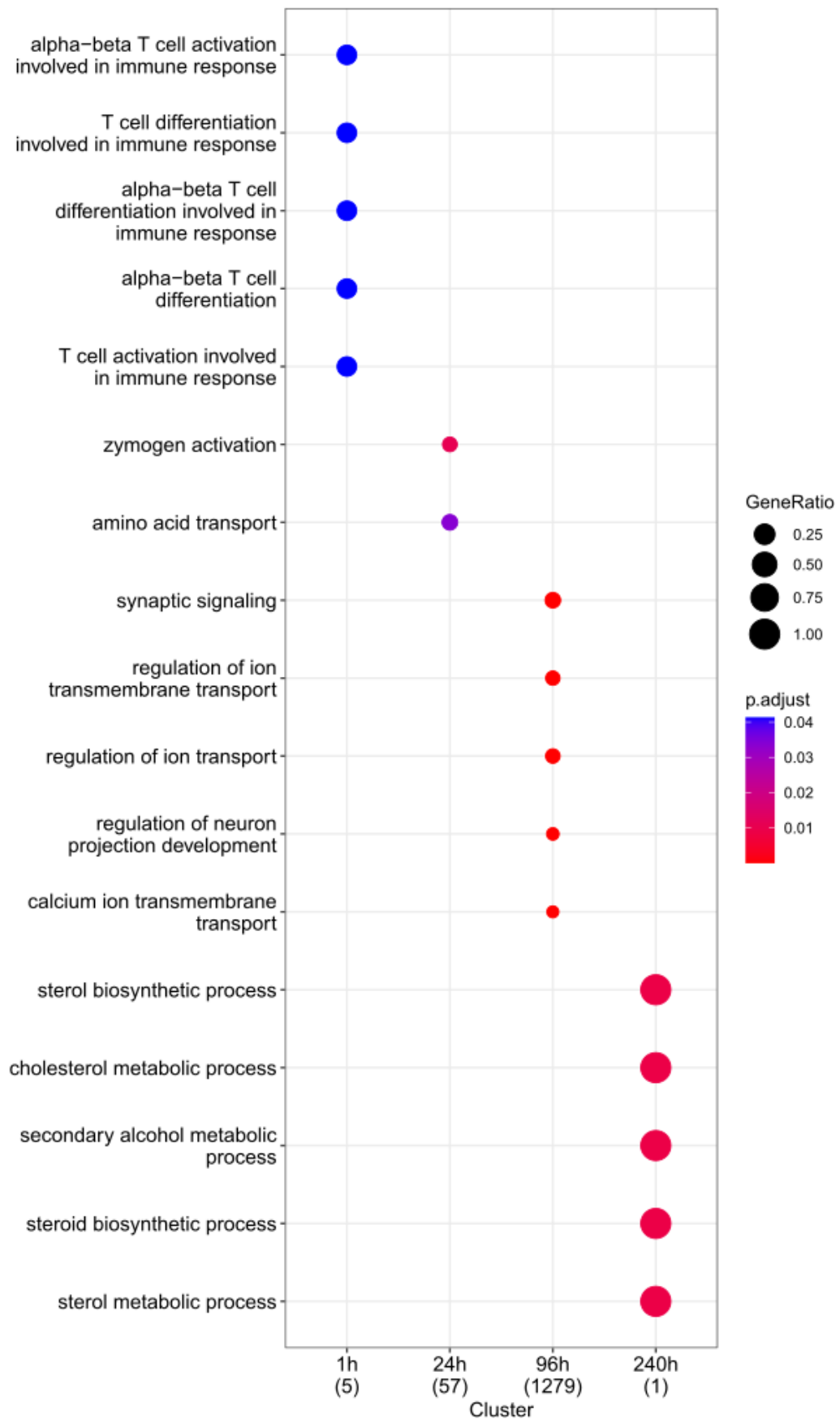


Figure S2 : Goterms enrichment of downregulated genes after stabwound injury



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GO:0023058	adaptation of signaling pathway	6E-04	5E-04	ENSDARG00000012610/ENSDARG00000038378/ENSDARG00000056511
GO:0045742	positive regulation of epidermal growth factor receptor signaling pathway	6E-04	5E-04	ENSDARG00000056791/ENSDARG00000102558/ENSDARG000000101984
GO:1901186	positive regulation of ERBB signaling pathway	6E-04	5E-04	ENSDARG00000056791/ENSDARG00000102558/ENSDARG000000101984
GO:0021982	pineal gland development	7E-04	6E-04	ENSDARG00000021201/ENSDARG00000068976/ENSDARG00000045904
GO:0042428	serotonin metabolic process	7E-04	6E-04	ENSDARG00000016494/ENSDARG00000057239/ENSDARG00000029432
GO:1901160	primary amino compound metabolic process	7E-04	6E-04	ENSDARG00000016494/ENSDARG00000057239/ENSDARG00000029432
GO:0001895	retina homeostasis	9E-04	8E-04	ENSDARG00000052035/ENSDARG00000030107/ENSDARG000000100397/ENSDARG00000045904
GO:0046530	photoreceptor cell differentiation	1E-03	1E-03	ENSDARG00000091756/ENSDARG00000102288/ENSDARG00000025504/ENSDARG00000076055/ENSDARG0000008306/ENSDARG00000045904
GO:0060221	retinal rod cell differentiation	1E-03	1E-03	ENSDARG00000091756/ENSDARG00000076055/ENSDARG00000045904
GO:0097164	ammonium ion metabolic process	2E-03	2E-03	ENSDARG00000016494/ENSDARG00000057239/ENSDARG00000029432
GO:0045744	negative regulation of G protein-coupled receptor signaling pathway	3E-03	2E-03	ENSDARG00000012610/ENSDARG00000038378/ENSDARG00000056511
GO:0009584	detection of visible light	3E-03	3E-03	ENSDARG00000020602/ENSDARG00000042529/ENSDARG00000102288
GO:0018298	protein-chromophore linkage	4E-03	4E-03	ENSDARG00000061515/ENSDARG00000111860/ENSDARG00000044672/ENSDARG00000052056
GO:0042058	regulation of epidermal growth factor receptor signaling pathway	6E-03	5E-03	ENSDARG00000056791/ENSDARG00000102558/ENSDARG000000101984
GO:0051606	detection of stimulus	6E-03	5E-03	ENSDARG00000061515/ENSDARG00000020602/ENSDARG00000042529/ENSDARG00000111860/ENSDARG00000102288/ENSDARG00000044672/ENSDARG00000052056
GO:0046189	phenol-containing compound	6E-03	5E-03	ENSDARG00000016494/ENSDARG00000057239/ENSDARG00000029432

GO:1901184	regulation of ERBB signaling pathway	6E-03	5E-03	ENSDARG00000056791/ENSDARG00000102558/ENSDARG000000101984
GO:0007173	epidermal growth factor receptor signaling pathway	1E-02	1E-02	ENSDARG00000056791/ENSDARG00000102558/ENSDARG000000101984
GO:0001754	eye photoreceptor cell differentiation	2E-02	1E-02	ENSDARG00000091756/ENSDARG00000076055/ENSDARG0000008306/ENSDARG00000045904
GO:0031623	receptor internalization	2E-02	1E-02	ENSDARG00000012610/ENSDARG00000038378/ENSDARG00000056511
GO:0052652	cyclic purine nucleotide metabolic process	2E-02	1E-02	ENSDARG00000026820/ENSDARG00000032838/ENSDARG00000025504
GO:0001894	tissue homeostasis	2E-02	1E-02	ENSDARG00000052035/ENSDARG00000030107/ENSDARG000000100397/ENSDARG00000045904
GO:0035270	endocrine system development	2E-02	1E-02	ENSDARG00000091756/ENSDARG00000068006/ENSDARG00000021201/ENSDARG00000068976/ENSDARG00000045904
GO:0009190	cyclic nucleotide biosynthetic process	2E-02	1E-02	ENSDARG00000026820/ENSDARG00000032838/ENSDARG00000025504
GO:0038127	ERBB signaling pathway	2E-02	1E-02	ENSDARG00000056791/ENSDARG00000102558/ENSDARG000000101984
GO:0060219	camera-type eye photoreceptor cell differentiation	2E-02	1E-02	ENSDARG00000091756/ENSDARG00000076055/ENSDARG00000045904
GO:0060042	retina morphogenesis in camera-type eye	2E-02	1E-02	ENSDARG00000091756/ENSDARG00000100397/ENSDARG00000087349/ENSDARG00000076055/ENSDARG00000045904
GO:0050908	detection of light stimulus involved in visual perception	2E-02	1E-02	ENSDARG00000042529/ENSDARG00000102288
GO:0050962	detection of light stimulus involved in sensory perception	2E-02	1E-02	ENSDARG00000042529/ENSDARG00000102288
GO:1903530	regulation of secretion by cell	2E-02	2E-02	ENSDARG00000037941/ENSDARG00000094889/ENSDARG00000114760/ENSDARG00000068006/ENSDARG00000059978
GO:0023061	signal release	2E-02	2E-02	ENSDARG00000037941/ENSDARG00000094889/ENSDARG00000114760/ENSDARG00000068006/ENSDARG00000059978
GO:0051046	regulation of secretion	2E-02	2E-02	ENSDARG00000037941/ENSDARG00000094889/ENSDARG00000114760/ENSDARG00000068006/ENSDARG00000059978

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GO:0018958	phenol-containing compound metabolic process	2E-02	2E-02	ENSDARG00000016494/ENSDARG00000057239/ENSDARG00000029432
GO:0048871	multicellular organismal homeostasis	3E-02	3E-02	ENSDARG00000052035/ENSDARG00000030107/ENSDARG000000100397/ENSDARG00000045904
GO:0043112	receptor metabolic process	3E-02	3E-02	ENSDARG00000012610/ENSDARG00000038378/ENSDARG00000056511
GO:0006898	receptor-mediated endocytosis	3E-02	3E-02	ENSDARG0000004459/ENSDARG00000012610/ENSDARG00000038378/ENSDARG00000056511
GO:0048592	eye morphogenesis	3E-02	3E-02	ENSDARG00000091756/ENSDARG000000100397/ENSDARG00000087349/ENSDARG00000076055/ENSDARG0000008306/ENSDARG00000045904
GO:0009187	cyclic nucleotide metabolic process	3E-02	3E-02	ENSDARG00000026820/ENSDARG00000032838/ENSDARG00000025504
GO:0090596	sensory organ morphogenesis	3E-02	3E-02	ENSDARG000000101980/ENSDARG00000091756/ENSDARG000000100397/ENSDARG00000087349/ENSDARG00000076055/ENSDARG0000008306/ENSDARG00000045904
GO:0060249	anatomical structure homeostasis	4E-02	3E-02	ENSDARG00000052035/ENSDARG00000030107/ENSDARG000000100397/ENSDARG00000045904
GO:0003407	neural retina development	4E-02	4E-02	ENSDARG00000091756/ENSDARG00000087349/ENSDARG00000076055/ENSDARG00000045904
GO:0007168	receptor guanylyl cyclase signaling pathway	4E-02	4E-02	ENSDARG00000026820/ENSDARG00000025504
GO:0016079	synaptic vesicle exocytosis	5E-02	4E-02	ENSDARG00000037941/ENSDARG00000094889/ENSDARG00000059978

3.2.3. Mains results & discussion

AXIS II: Common and differential features of IR response compared to regenerative neurogenesis induced by neuroinflammation

Question 1: What can we learn from transcriptomic analyses after wounding adult zebrafish telencephalon?

We performed stabwound injury in the right telencephalon of adult zebrafish and analyzed the transcriptional response at several time points post lesion: 0 hpl, 1 hpl, 3 hpl, 10 hpl, 24 hpl, 96 hpl, 240 hpl. We could confirm results from previous studies such as the initiation of apoptosis process, inflammatory response, activation of the immune system or cell proliferation and demonstrated that some of these processes were regulated as soon as 1hpl. Interestingly, we found modulation of ion transport and axonogenesis only described in retina or spinal cord regeneration until now. These two processes could be linked since spinal cord injury relies, among other processes, on the rapid elevation of intra-axonal Ca²⁺ (Tsata and Wehner, 2021).

Question 2: What are the specificities of radiation induced effects in the brain?

One of the main effects on chronic low to moderate dose rates of IR was the enrichment in visual perception, retinoid binding and phototransduction and we could detect the same results at 1 hpl, 3 hpl and 96 hpl. Additionally, we could observe the upregulation of genes (i.e. *tph1a*, *tph2*, *aanat2* and *asmt*) involved in melatonin synthesis and the expression of *aanat2* in the telencephalon. Together, these results strongly suggests that cells expressing photoreceptive molecules located in the telencephalon are targeted by both stabwound injury and chronic low to moderate dose rates of IR. In the preoptic area of larval zebrafish, this photosensitive region contains an ancient population of sensory neurons, suggested to be specified by *otpa*, and these neurons mediate behavioral responses to light (phototaxis) (Fernandes et al., 2012).

Since we observed an upregulation of several genes involved in the process of neurogenesis in Axis I, we checked if the same genes were dysregulated after stabwound injury (**Table 3**). Most genes upregulated after 5 mGy.h⁻¹ radiation were also upregulated at 1 hpl, 3 hpl and even 96 hpl except for some genes such as *crx* and *dpysl3*. Why these

genes are impacted, and what role they could play during regeneration remain to be determined. Effects on axons, ion transport and cortisol secretion were detected during telencephalon regeneration and these processes were also impacted after irradiation (e.g. axonal guidance: *sema3d*, *wnt4*; ion transport: *cnga1a*, *fthl27*). These elements are in favour for the activation of neurogenesis after irradiation. Even if we did not observe any inflammatory process after irradiation, we cannot exclude that this phenomenon was previously activated and led to neurogenesis.

Table 3 – Fold change and adjusted p-values (fdr) of genes with function in neurogenesis after irradiation and after induction of brain regeneration by stab wound. FC: Fold Change. FDR: False discovery rate.

gene name		0.5 mGy.h ⁻¹	5 mGy.h ⁻¹	1 hpl	3 hpl	10 hpl	24 hpl	96 hpl	240 hpl
arl3l1	FC	5,71	5,84	6,79	6,47	x	x	5,57	4,68
	FDR	3,12E-14	5,22E-18	2,76E-18	5,75E-16			2,15E-12	2,57E-08
arl3l2	FC	1,47	2,22	1,92	2,03	x	x	x	x
	FDR	0,001	5,45E-11	1,73E-05	1,68E-06				
atp1b2b	FC	2,90	2,86	2,69	2,29	x	x	x	x
	FDR	0,0001	1,02E-05	0,02	0,02				
crx	FC	8,40	7,41	x	x	x	x	x	x
	FDR	9,50E-06	4,81E-05						
dpysl3	FC	x	-0,28	x	x	x	x	x	x
	FDR		0,02						
en1b	FC	2,06	x	x	x	x	x	x	x
	FDR	0,04							
faimb	FC	2,94	3,12	2,17	1,85	x	x	1,49	x
	FDR	6,85E-09	1,47E-12	0,0003	0,003			0,007	
foxd3	FC	x	3,50	x	x	x	x	5,87	x
	FDR		0,04					0,02	
gucy2f	FC	4,43	4,77	5,23	5,31	x	x	3,32	3,31
	FDR	5,23E-08	1,88E-11	4,05E-17	4,13E-18			3,46E-08	1,84E-06
insm1a	FC	1,65	1,90	1,98	2,02	x	x	1,73	1,24
	FDR	1,19E-06	3,66E-11	8,63E-07	2,73E-07			4,01E-06	0,04
klf8	FC	x	-0,2836181	x	x	x	x	-0,55	x
	FDR		0,03					3,29E-05	
lhx4	FC	4,86	5,34	6,94	7,36	x	x	5,72	6,13
	FDR	4,39E-06	2,92E-09	1,04E-23	1,98E-24			1,52E-16	1,89E-13

nav1	FC		2,62					-1,16	
	FDR	x	0,03	x	x	x	x	1,31E-05	x
nexn	FC		1,44	2,17					
	FDR	x	0,03	3,58E-06	x	x	x	x	x
nrn1lb	FC	6,08	6,11						
	FDR	0,03	0,01	x	x	x	x	x	x
plxnb3	FC		2,63			1,03		-1,00	
	FDR	x	0,02	x	x	0,05	x	0,01	x
ppp1r9a	FC		3,37					-1,23	
	FDR	x	0,001	x	x	x	x	5,68E-06	x
prom1b	FC		1,21	2,23	1,75			1,13	
	FDR	x	0,03	0,0001	0,003	x	x	0,04	x
rdh5	FC	5,33	5,89	7,62	8,35			5,77	
	FDR	1,17E-09	7,04E-15	7,76E-09	4,87E-10	x	x	3,51E-06	
rhogd	FC	3,07						1,88	
	FDR	0,002	x	x	x	x	x	0,05	x
rpgrip1	FC	4,48	5,15	6,25	6,57			5,34	6,58
	FDR	4,39E-06	5,28E-10	4,58E-08	1,00E-07	x	x	3,00E-07	0,01
si:ch73-119p20	FC	-2,02	-2,04						
	FDR	0,02	0,002	x	x	x	x	x	x
unc119.2	FC	5,30	5,65	5,90	5,19			4,41	3,56
	FDR	5,94E-13	5,08E-18	7,17E-08	2,61E-06	x	x	4,57E-05	0,03
znf365	FC		1,65					-1,21	
	FDR	x	0,05	x	x	x	x	6,02E-07	x

Since we identified potential markers of exposure to IR, we assessed if we could detect the same responses after stabwoud injury. Herein, we focused on the expression levels of genes involved in neurotransmission and neurohormones at D05, D5, 1hpl and 3 hpl (Figure 22).

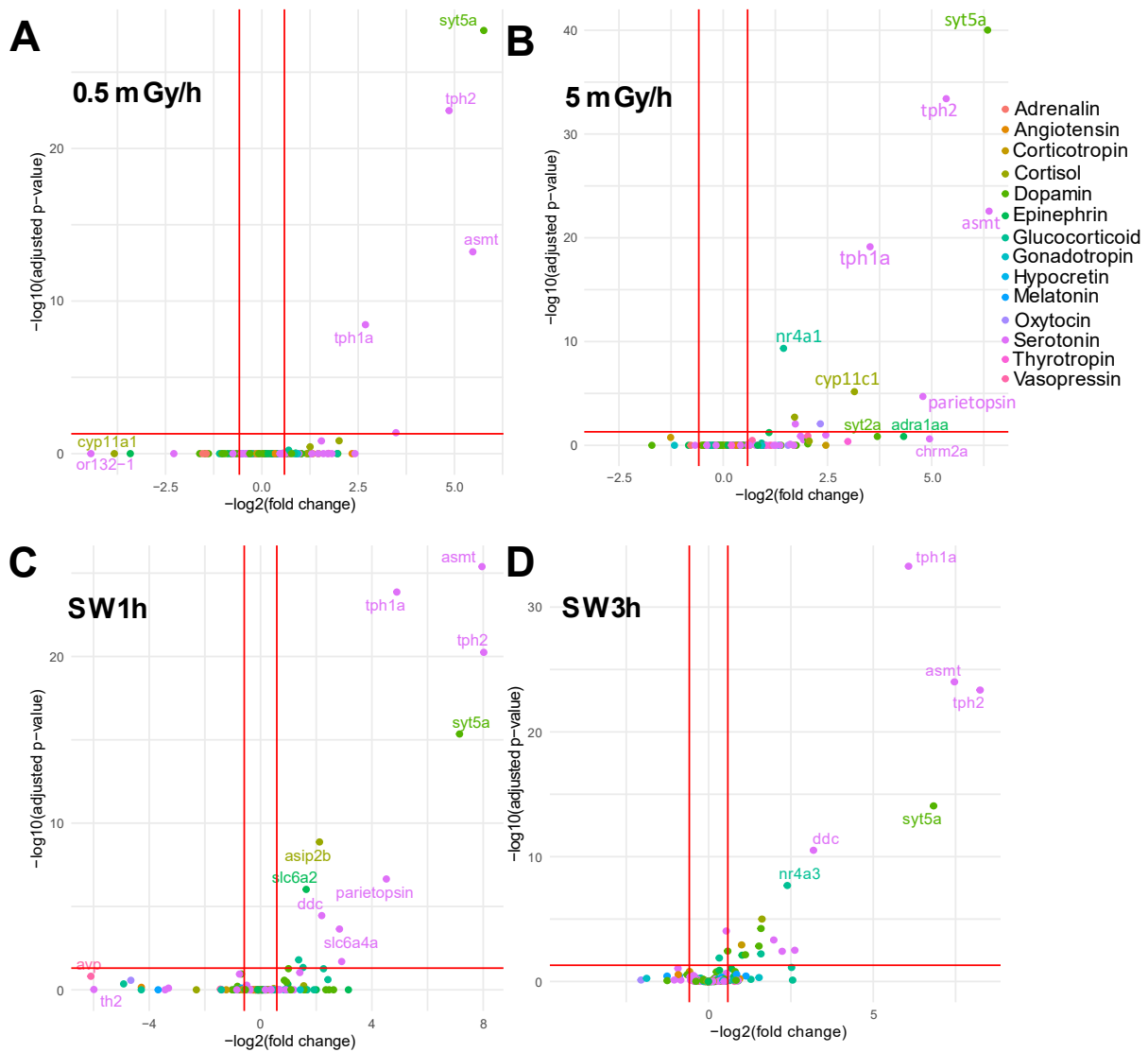


Figure 22 - Volcano plot of differentially expressed genes after irradiation and stab wound injury (SW) with function in neurotransmission or brain neurohormones.

From that analysis, we could detect some shared molecular markers between brain regeneration and response to chronic exposure to IR including upregulation of *sl6a2* involved in epinephrin and norepinephrine transport, *asip2b* involved in cortisol secretion, *tph1a*, *tph2* that act on serotonin synthesis, *ddc* that play a role in serotonin and dopamine synthesis, and *syt5a* proposed to have a role in dopamine release. Interestingly, oxytocin nor arginine vasopressin were never modulated during brain regeneration after stab wound, which could thus suggest that they are specific molecular markers of chronic exposure to IR (5 mGy.h⁻¹).

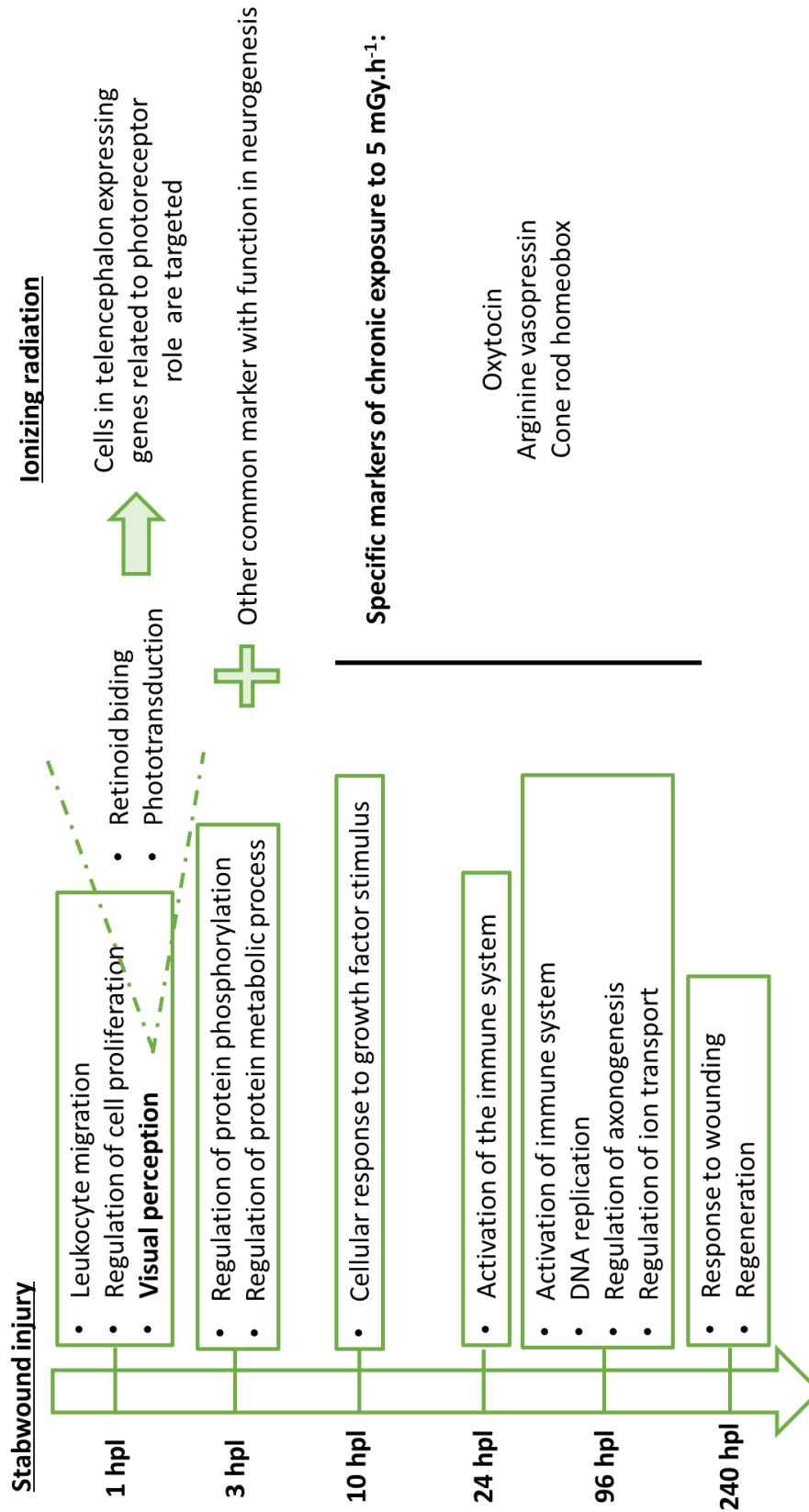


Figure 23 - Schematic overview resuming commonalities and differences between lesion after stabwound and chronic low to moderate dose rates of IR

4. General conclusion and perspectives

Outcomes

The initial aim of my PhD work was to investigate the molecular mechanisms involved after exposure to chronic low to moderate dose rates of ionizing radiation and how they are related to higher biological scales. Our results provide elements of answer and prospective clues for further investigations. More specifically:

- We identified a dose rate response relationship with the number of dysregulated genes.
- We identified effects on behavior only in female's which displayed a stress response, a reduced locomotion and social behavior.
- Acetylcholine was not involved in these changes in behavior, however serotonin and dopamine constitute good candidates.
- We highlighted that oxytocin and arginine vasopressin can play a role in stress and social behavior at 5 mGy.h⁻¹.
- We characterized a response to chronic exposure and low and moderate dose rates of IR which is distinct from the effects described after acute and high exposure (DNA damage, oxidative stress, inflammation, reduction in neurogenesis).
- The comparison between irradiated fish and the stabwound injury highlighted similarities between the two responses. The molecular data suggested that neurogenesis might be activated after chronic irradiation and highlighted the activation of genes with photoreceptor role located in the telencephalon.

Responses to chronic low to moderate dose rates of IR in the adult zebrafish brain

Overall, the possible underlying mechanisms causing stress response and reduced sociability might be due to the alteration of serotonin and dopamine neurotransmission at 0.5 mGy.h⁻¹ and 5 mGy.h⁻¹. At 5mGy.h⁻¹, additional molecular actors might be involved. Indeed, oxytocin through its receptor *oxtrl* could perturb social behavioral response and might act in synergy with arginine vasopressin and also interact with the HPI stress axis

causing the increased stress response. However, we cannot exclude that oxytocin is also upregulated at 0.5 mGy.h⁻¹ but not detected by transcriptomics as we pooled both males and females telencephalon. Surprisingly, the upregulation of enzymes involved in melatonin was also detected at 0.5 mGy.h⁻¹ and 5 mGy.h⁻¹. Melatonin and oxytocin were shown to both play a role as antioxidants. Melatonin has been documented as a direct free radical scavenger, and *in vitro* as well as *in vivo* studies have confirmed that melatonin can protect from toxic effects of ionizing radiation (Ahlers et al., 1997; Badr et al., 1999; Blickenstaff et al., 1994; Kaya et al., 1999; Meltz et al., 1999).

Additionally, a recent study from Igarashi and colleagues demonstrated the neuroprotective effects of exogenous oxytocin administration by attenuating cognitive dysfunctions, DNA damage and preventing the breakdown of chloride ion concentration in nerve cells after cranial radiation therapy in young mice (Igarashi et al., 2022). Consequently, our results might demonstrate that after chronic exposure to low to moderate dose rates of ionizing radiation the oxidative stress can be directly compensated by melatonin and oxytocin over expression (our transcriptomic results). Additionally, cortisol might act through its anti-inflammatory role and be able to overpast the initial neuroinflammation state.

Other targets of ionizing radiation

In the context of this project, we focused on the molecular changes in brain and the consequences in behavior. As we performed the irradiation of whole zebrafish it is possible that other organs could have been impacted and contribute indirectly to the phenotypes we observed.

For example, the lateral line is a sensory system used to detect changes in water flows which facilitates biological behaviors such as foraging and escaping. The lateral line system is composed of neuromast positioned as a line along the surface of the body of fish as superficial lines that cover the head and body. The modulation of the sensory input occurs via the connection of hair cells (in contact to the water flow) to excitatory and inhibitory efferent neurons located in the brain. Thus, fish can detect movements in water through this lateral line and guide fish interactions with shoal members (Gerlai, 2014; Manuel et al., 2021). It has already been demonstrated that the visual system is not required when swimming in group. Indeed, blind fish can use their lateral line to swim properly in group by sensing low frequency waves (Gregson and De Perera, 2007;

Partridge and Pitcher, 1980). Additionally, the lateral line was demonstrated to be targeted by ionizing radiation since a loss of hair cells and an inhibition of cell proliferation of neuromasts was detected in zebrafish larvae. However, from our results of the shoaling test, we did not detect any changes in the interactions between fishes which suggest that the lateral line was not impacted by ionizing radiation or that defective cells in the lateral lines were able to regenerate rapidly enough (Li et al., 2018).

It has also been demonstrated that metabolic processes can be targeted by ionizing radiation in *Caenorhabditis elegans* (Dufourcq-Sekatcheff et al., 2021; Guédon et al., 2021) with effects on lipid metabolism and transport, including fatty acid metabolic process, lipid transport, sphingolipid biosynthetic processes and lipid oxidation. Such effects were detected also after low -dose of ionizing radiation in the mouse brain with dysregulation of genes involved in fatty acid/lipid metabolism (Yin et al., 2003). Exposure of IR at dose rate greater or equal to 0.5 mGy.h⁻¹ altered the regulation of glycolysis in mitochondria and energetic metabolism in 6 hpf embryo zebrafish (Murat El Houdigui et al., 2020). Moreover, in zebrafish, exposure to Fluorene-9-bisphenol caused defects in 1,25(OH)₂D₃ metabolic pathway through downregulation of *cyp2r1*, which further damaged *pgc1a*-mediated fatty acid oxidation and mitochondrial function, resulting in lipid accumulation and reduced locomotive activity of larvae suggesting a direct link between these processes (Zhang et al., 2022). In our experimental data, we found the enrichment of fat-soluble vitamin metabolic process with upregulation of genes as *cyp2r1* or *cyp24a1*. Consequently, these dysregulated genes could impact lipid and fatty acid metabolism and might at least in part be the cause of hypolocomotion. However, more data at the level of muscle physiology are required to assess properly this effect.

Another interesting system is the gut-brain axis since links between the enteric microbiota and brain function have been demonstrated (Al-Asmakh et al., 2012; Cryan and Dinan, 2012; O'Sullivan et al., 2011). Indeed, modifications on the enteric microbiota could affect stress associated anxiety-like and depressive-like behavior (Bertotto et al., 2020; Lee et al., 2021) . For example, in adult zebrafish, the supplementation with *L. plantarum* induced a subtle alteration in intestinal microbiota and caused a significant reduction in stress response detected by the novel tank test (Davis et al., 2016). Additionally, using rodents as model organisms, multiple studies have also implicated the microbiota in regulation of serotonin signaling and metabolism in rodents (Clarke et al., 2014, 2013; Yano et al., 2015). In zebrafish, the feeding with probiotic strain *L. rhamnosus*

induced significantly differences in the shoaling pattern and exploratory behavior and suggested a link with the altered expression of brain derived neurotrophic factor and genes related to serotonin metabolism and signaling (*tph1a*, *tph1b* and *tph2*) (Bertotto et al., 2020). Interestingly, one study detected changes in the composition of the gut microbiota after exposure to gamma radiation (Cobalt 60) with doses varying from 0.05 Gy (10 doses administrated) to 0.5 Gy (1 dose administrated) (Liu et al., 2019). Herein, we cannot exclude that the dysregulation of serotonin in our experimental studies is not a direct consequence from modulations coming from the microbiota of our irradiated zebrafish.

Perspectives and limits

Limits of our experimental setting

- **Irradiation settings**

We focused on the effects of the brain however we did irradiate the whole body of the fish. It could also have been interesting to set an experiment where only the brain is irradiated so the observed effects would have directly ensued from the irradiation of the brain. However, even if this technique is available within IRSN (SARRP) it is not applicable for chronic exposure since the immobility of the fish is required and consequently bias such as the induction of a stress response would have complexify the interpretation of the results. Furthermore, whole body irradiation is the favoured exposure scenario in the context of environmental exposure to IR, as exemplified for biota present in Chernobyl or Fukushima.

- **Transcriptomic studies**

The great benefit of transcriptomic studies is the global approach that sets the dysregulation of expression of genes and clusters them in known processes or signaling cascades. However, this strategy is not sufficient to identify which functional pathway do disturb the behavior of fish in our context of exposure and thus, additional studies are needed such as blocking oxytocin receptors (*oxtrl* and *oxtr*) or using zebrafish oxytocin receptor knockout line or inactivating HPI axis by treating with CRH receptor antagonist associated to radiation exposure. Also, it is hard to conclude with certainty that the observed dysregulation of genes is inducing the behavioral modifications since we have

no clue on the regulation of the feedback regulating loops and possibly the individual behavioral responses are causing dysregulation of several expression genes.

Additionally, since we only observed modulation of behavioral patterns among females, it would have been more appropriate to separate the sexes when pooling brains for RNA sequencing. This is a challenging argument because much more individuals would have been needed, more workforce and the costs of the experiment would have doubled.

Finally, the interpretation of transcriptomics data might be biased by the lack of comprehensive gene function annotations for zebrafish genes. Indeed, many genes were found to be involved in visual perception however RNA *in situ* hybridization confirmed the expression in the telencephalon in the VZ. A good example is *crx*, which is currently annotated in the zebrafish and human genome as a gene involved in visual perception. The expression of this gene in the VZ of the ventral telencephalon confirms that this gene is expressed in neural progenitors, but the function of *crx* in this tissue is currently unknown. Co-staining with these RNAs probes and specific markers of progenitor cells or differentiated neurons could help to precisely define these types of cells and then functional studies could be useful to identify their role in the telencephalon.

- **Behavioral studies**

Focusing on our transcriptomic results, we could identify enrichment in visual perception and phototransduction and an upregulation of genes that code for enzymes involved in melatonin synthesis. As melatonin is well known to induce sleep behavior, as an integrator of the circadian rhythm (Lima-Cabello et al., 2014), it would be interesting to evaluate the effects of IR on circadian rhythm though sleep like state behavior (Chiu and Prober, 2013; Singh et al., 2013). Another aspect could be to perform optokinetic test to verify if vision can be affected (Li et al., 2012; Mueller and Neuhaus, 2010). An impact on these types of behavior would probably affect predatory avoidance and/or foraging.

Going deeper into this project

- **What is already planned**

It is well known that after acute exposure to ionizing radiation, DNA damage and apoptotic process are activated. We have evaluated neuronal death using Fluorojade C staining but no clear effect was detected on adult brain vibratome section. Other markers are under completion including immunostaining to characterize apoptosis using active Caspase 3, double strand breaks DNA damage using γ -H2AX immunostaining and immunostaining against PCNA and HuC/D to identify potential effects on proliferation and differentiated neurons (Roy et al., 2009). To better characterize the process of neurogenesis, another useful technique regarding our experimental setup could be BrdU tracing to follow cellular proliferation and migration of neurons and thus will be very informative. Finally, performing proteomics or western blots on specific markers would be very informative and could be linked to the changes in behavior.

Serotonin and dopamine are upregulated based on our transcriptional studies. We thus, aim at dosing neurotransmitters and their metabolites in the brain, using HPLC-MS, through the collaboration with Christina Dallas from the Medical School, National & Kapodistrian University of Athens. Since serotonin is well known for its role in aggressivity, we performed the mirror induced aggressivity test. The results are currently being analyzed. Finally, we planned to dose whole body cortisol by radioimmunoassay by collaborating with Thierry Charlier (co-director) at IRSET.

- **Potential further investigations**

- Going deeper into the mechanisms of action

As suggested by the upregulation of genes involved in melatonin synthesis, the pineal gland might be a target organ of IR and it could be interesting to assess its role in the behavioral pattern observed. For example, the ablation of pineal photoreceptor cells has already been explored to investigate the effects on the circadian rhythms of behavioral visual sensitivity in zebrafish and showed that retinal photoreceptor cells are sufficient to entrain the circadian rhythms of behavioral visual sensitivity while cellular signals from pineal photoreceptor cells are needed for maintaining the rhythms (Li et al., 2012). Additionally, I have tried to explore the expression of genes in the pineal gland by

RNA *in situ* hybridization, but vibratome section was not suitable since the pineal gland was not always present. Instead, it would be appropriate to perform cryosection on whole head of the fish to maintain the integrity of this organ.

We also proposed that the habenula might be involved in the behavioral modifications that we observed, but I did not have time to explore this possibility with additional studies. I suggest that by specifically interfering with dorsal habenula (that plays a role in fear and anxiety) we could have a better understanding of its involvement. This manipulation has already been performed by applying the transgenic tetanus neurotoxin technique to selectively silence neurotransmission of the dorsal habenula and we could thus perform it to evaluate zebrafish behavior after radiation (Bühler and Carl, 2021; Okamoto et al., 2012).

- Broadening the scope

We could focus on the transgenerational effects to determine if molecular responses and behavioral modifications are worsened in the next generations. Indeed, ionizing radiation were already demonstrated to induce transgenerational effects of DNA methylation in zebrafish when irradiated to 8.7 mGy.h^{-1} for 27 days (Kamstra et al., 2018).

Our results reflect the early time effects of ionizing radiation however it would be of great interest to complete these studies by analyzing the long-term effects. We could propose a kinetic approach at several time points post irradiation for transcriptional studies associated with behavioral tests. Finally, data from the contaminated area (in aquatic population) such as transcriptomic and behavioral effects could complete the long-term effects of our work to accurately assess the consequences on individuals from post nuclear accidents and from all their expositions to diverse contaminants.

REFERENCES

- 14.2: Types of Radioactivity- Alpha, Beta, and Gamma Decay [WWW Document], 2022. .
Chem. Libr. URL
https://chem.libretexts.org/Courses/Madera_Community_College/MacArthur_Chemistry_3A_v_1.2/14%3A_Radioactivity_and_Nuclear_Chemistry/14.02%3A_Types_of_Radioactivity-_Alpha_Beta_and_Gamma_Decay (accessed 10.18.22).
- Achanta, P., Capilla-Gonzalez, V., Purger, D., Reyes, J., Sailor, K., Song, H., Garcia-Verdugo, J.M., Gonzalez-Perez, O., Ford, E., Quinones-Hinojosa, A., 2012. Subventricular zone localized irradiation affects the generation of proliferating neural precursor cells and the migration of neuroblasts. *Stem Cells* 30, 2548–2560.
- Achanta, P., Fuss, M., Martinez Jr, J.L., 2009. Ionizing radiation impairs the formation of trace fear memories and reduces hippocampal neurogenesis. *Behav. Neurosci.* 123, 1036.
- Acharya, M.M., Lan, M.L., Kan, V.H., Patel, N.H., Giedzinski, E., Tseng, B.P., Limoli, C.L., 2010. Consequences of ionizing radiation-induced damage in human neural stem cells. *Free Radic. Biol. Med.* 49, 1846–1855.
<https://doi.org/10.1016/j.freeradbiomed.2010.08.021>
- Adams, K.L., Gallo, V., 2018. The diversity and disparity of the glial scar. *Nat. Neurosci.* 21, 9–15.
- Adolf, B., Chapouton, P., Lam, C.S., Topp, S., Tannhäuser, B., Strähle, U., Götz, M., Bally-Cuif, L., 2006. Conserved and acquired features of adult neurogenesis in the zebrafish telencephalon. *Dev. Biol.* 295, 278–293.
- Ahlers, I., Solar, P., Ahlersova, E., Kassayova, M., Smajda, B., 1997. The influence of melatonin on metabolic changes in female rats induced by continuous irradiation and/or administration of 7, 12-dimethylbenz/a/anthracene. *Neoplasma* 44, 253–257.
- Al-Asmakh, M., Anuar, F., Zadjali, F., Rafter, J., Pettersson, S., 2012. Gut microbial communities modulating brain development and function. *Gut Microbes* 3, 366–373.
- Alunni, A., Bally-Cuif, L., 2016. A comparative view of regenerative neurogenesis in vertebrates. *Development* 143, 741–753.
- Alunni, A., Krecsmarik, M., Bosco, A., Galant, S., Pan, L., Moens, C.B., Bally-Cuif, L., 2013. Notch3 signaling gates cell cycle entry and limits neural stem cell amplification in the adult pallium. *Development* 140, 3335–3347.
- Alvarez-Buylla, A., Lim, D.A., 2004. For the long run: maintaining germinal niches in the adult brain. *Neuron* 41, 683–686.
- Amo, R., Fredes, F., Kinoshita, M., Aoki, R., Aizawa, H., Agetsuma, M., Aoki, T., Shiraki, T., Kakinuma, H., Matsuda, M., 2014. The habenulo-raphe serotonergic circuit encodes an aversive expectation value essential for adaptive active avoidance of danger. *Neuron* 84, 1034–1048.
- Anacker, C., Cattaneo, A., Luoni, A., Musaelyan, K., Zunszain, P.A., Milanese, E., Rybka, J., Berry, A., Cirulli, F., Thuret, S., 2013a. Glucocorticoid-related molecular signaling pathways regulating hippocampal neurogenesis. *Neuropsychopharmacology* 38, 872–883.

- Anacker, C., Cattaneo, A., Musaelyan, K., Zunszain, P.A., Horowitz, M., Molteni, R., Luoni, A., Calabrese, F., Tansey, K., Gennarelli, M., 2013b. Role for the kinase SGK1 in stress, depression, and glucocorticoid effects on hippocampal neurogenesis. *Proc. Natl. Acad. Sci.* 110, 8708–8713.
- Anand, S.K., Sahu, M.R., Mondal, A.C., 2021. Induction of oxidative stress and apoptosis in the injured brain: potential relevance to brain regeneration in zebrafish. *Mol. Biol. Rep.* 48, 5099–5108.
- AncientPages.com, 2017. On This Day In History: Discovery Of X-Rays By Wilhelm Roentgen Reported - On Jan 5, 1896 [WWW Document]. *Anc. Pages*. URL <https://www.ancientpages.com/2017/01/05/on-this-day-in-history-discovery-of-x-rays-by-wilhelm-roentgen-reported-on-jan-5-1896/> (accessed 10.18.22).
- Andersson, P., Garnier-Laplace, J., Beresford, N.A., Copplestone, D., Howard, B.J., Howe, P., Oughton, D., Whitehouse, P., 2009. Protection of the environment from ionising radiation in a regulatory context (PROTECT): proposed numerical benchmark values. *J. Environ. Radioact.* 100, 1100–1108.
- Antonelli, C., Basso, M., Beguin-Leprieur, M., Boyer, P., Blanchart, P., Huet, C., Heller, M., Masson, O., Mathieu, G., Metivier, J.-M., 2021. Assessment of the radiological situation of the French environment from 2018 to 2020.
- Armant, O., Gombeau, K., Murat El Houdigui, S., Floriani, M., Camilleri, V., Cavalie, I., Adam-Guillermin, C., 2017. Zebrafish exposure to environmentally relevant concentration of depleted uranium impairs progeny development at the molecular and histological levels. *PLoS One* 12, e0177932.
- Armant, O., März, M., Schmidt, R., Ferg, M., Diotel, N., Ertzer, R., Bryne, J.C., Yang, L., Baader, I., Reischl, M., 2013. Genome-wide, whole mount in situ analysis of transcriptional regulators in zebrafish embryos. *Dev. Biol.* 380, 351–362.
- Averbeck, D., Candéias, S., Chandna, S., Foray, N., Friedl, A.A., Haghdoost, S., Jeggo, P.A., Lumniczky, K., Paris, F., Quintens, R., 2020. Establishing mechanisms affecting the individual response to ionizing radiation. *Int. J. Radiat. Biol.* 96, 297–323.
- Ayala, A., Muñoz, M.F., Argüelles, S., 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid. Med. Cell. Longev.* 2014.
- Ayari, B., El Hachimi, K.H., Yanicostas, C., Landoulsi, A., Soussi-Yanicostas, N., 2010. Prokineticin 2 expression is associated with neural repair of injured adult zebrafish telencephalon. *J. Neurotrauma* 27, 959–972.
- Azizova, T.V., Bannikova, M.V., Grigoryeva, E.S., Rybkina, V.L., Hamada, N., 2020. Occupational exposure to chronic ionizing radiation increases risk of Parkinson's disease incidence in Russian Mayak workers. *Int. J. Epidemiol.* 49, 435–447. <https://doi.org/10.1093/ije/dyz230>
- Badr, F., El Habit, O., Harraz, M., 1999. Radioprotective effect of melatonin assessed by measuring chromosomal damage in mitotic and meiotic cells. *Mutat. Res. Toxicol. Environ. Mutagen.* 444, 367–372.
- Bai, G., Sheng, N., Xie, Z., Bian, W., Yokota, Y., Benezra, R., Kageyama, R., Guillemot, F., Jing, N., 2007. Id sustains Hes1 expression to inhibit precocious neurogenesis by releasing negative autoregulation of Hes1. *Dev. Cell* 13, 283–297.
- Barbosa, J.S., Ninkovic, J., 2016. Adult neural stem cell behavior underlying constitutive and restorative neurogenesis in zebrafish. *Neurogenesis Austin Tex* 3, e1148101. <https://doi.org/10.1080/23262133.2016.1148101>

- Barbosa, J.S., Sanchez-Gonzalez, R., Di Giaimo, R., Baumgart, E.V., Theis, F.J., Götz, M., Ninkovic, J., 2015. Live imaging of adult neural stem cell behavior in the intact and injured zebrafish brain. *Science* 348, 789–793.
- Baumgart, E.V., Barbosa, J.S., Bally-cuif, L., Götz, M., Ninkovic, J., 2012. Stab wound injury of the zebrafish telencephalon: a model for comparative analysis of reactive gliosis. *Glia* 60, 343–357.
- Bayounes, O., Souffrin, C., Meunier, D., 2013. Le Radium: découverte, utilisation et danger.
- Becker, C.G., Becker, T., 2007. Growth and pathfinding of regenerating axons in the optic projection of adult fish. *J. Neurosci. Res.* 85, 2793–2799.
- Becker, C.G., Schweitzer, J., Feldner, J., Schachner, M., Becker, T., 2004. Tenascin-R as a repellent guidance molecule for newly growing and regenerating optic axons in adult zebrafish. *Mol. Cell. Neurosci.* 26, 376–389.
- Becker, T., Becker, C.G., 2014. Axonal regeneration in zebrafish. *Curr. Opin. Neurobiol.* 27, 186–191.
- Becker, T., Wullmann, M.F., Becker, C.G., Bernhardt, R.R., Schachner, M., 1997. Axonal regrowth after spinal cord transection in adult zebrafish. *J. Comp. Neurol.* 377, 577–595.
- Belka, C., Budach, W., Kortmann, R., Bamberg, M., 2001. Radiation induced CNS toxicity—molecular and cellular mechanisms. *Br. J. Cancer* 85, 1233–1239.
- Bely, A.E., Nyberg, K.G., 2010. Evolution of animal regeneration: re-emergence of a field. *Trends Ecol. Evol.* 25, 161–170.
- Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B Methodol.* 57, 289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>
- Bertotto, L.B., Catron, T.R., Tal, T., 2020. Exploring interactions between xenobiotics, microbiota, and neurotoxicity in zebrafish. *Neurotoxicology* 76, 235–244.
- Bier, E., De Robertis, E., 2015. EMBRYO DEVELOPMENT. BMP gradients: a paradigm for morphogen-mediated developmental patterning. *Science* 348: aaa5838.
- Biographie - Henri Becquerel, n.d.
- Bladen, C.L., Lam, W.K., Dynan, W.S., Kozlowski, D.J., 2005. DNA damage response and Ku80 function in the vertebrate embryo. *Nucleic Acids Res.* 33, 3002–3010.
- Blickenstaff, R.T., Brandstadter, S.M., Reddy, S., Witt, R., 1994. Potential radioprotective agents. 1. Homologs of melatonin. *J. Pharm. Sci.* 83, 216–218.
- Blom, E., Coetzer, W.G., Schneider, S.-R., Grobler, J.P., 2022. The phylogenetic position of zebrafish (*Danio rerio*) from south african pet shops. *Mol. Biol. Rep.* 1–10.
- Boron, W., 2017. Organization of the nervous system.
- Braida, D., Donzelli, A., Martucci, R., Capurro, V., Busnelli, M., Chini, B., Sala, M., 2012. Neurohypophyseal hormones manipulation modulate social and anxiety-related behavior in zebrafish. *Psychopharmacology (Berl.)* 220, 319–330.
- Brown, C., Markula, A., Laland, K., 2003. Social learning of prey location in hatchery-reared Atlantic salmon. *J. Fish Biol.* 63, 738–745.
- Brown, D.L., Kao, W.W., Greenhalgh, D.G., 1997. Apoptosis down-regulates inflammation under the advancing epithelial wound edge: delayed patterns in diabetes and improvement with topical growth factors. *Surgery* 121, 372–380.
- Bühler, A., Carl, M., 2021. Zebrafish Tools for Deciphering Habenular Network-Linked Mental Disorders. *Biomolecules* 11, 324.
- Cacialli, P., D'angelo, L., Kah, O., Coumailleau, P., Gueguen, M., Pellegrini, E., Lucini, C., 2018. Neuronal expression of brain derived neurotrophic factor in the injured telencephalon of adult zebrafish. *J. Comp. Neurol.* 526, 569–582.

- Caldwell, H.K., 2017. Oxytocin and vasopressin: powerful regulators of social behavior. *The Neuroscientist* 23, 517–528.
- Caldwell, L.J., Davies, N.O., Cavone, L., Mysiak, K.S., Semenova, S.A., Panula, P., Armstrong, J.D., Becker, C.G., Becker, T., 2019. Regeneration of Dopaminergic Neurons in Adult Zebrafish Depends on Immune System Activation and Differs for Distinct Populations. *J. Neurosci.* 39, 4694. <https://doi.org/10.1523/JNEUROSCI.2706-18.2019>
- Cameron, D.A., Gentile, K.L., Middleton, F.A., Yurco, P., 2005. Gene expression profiles of intact and regenerating zebrafish retina. *Mol Vis* 11, 91.
- Cantabella, E., Camilleri, V., Cavalie, I., Dubourg, N., Gagnaire, B., Charlier, T.D., Adam-Guillermin, C., Cousin, X., Armant, O., 2022. Revealing the Increased Stress Response Behavior through Transcriptomic Analysis of Adult Zebrafish Brain after Chronic Low to Moderate Dose Rates of Ionizing Radiation. *Cancers* 14, 3793.
- Cavone, L., McCann, T., Drake, L.K., Aguzzi, E.A., Oprişoreanu, A.-M., Pedersen, E., Sandi, S., Selvarajah, J., Tsarouchas, T.M., Wehner, D., 2021. A unique macrophage subpopulation signals directly to progenitor cells to promote regenerative neurogenesis in the zebrafish spinal cord. *Dev. Cell* 56, 1617–1630.
- Ceci, M., Mariano, V., Romano, N., 2019. Zebrafish as a translational regeneration model to study the activation of neural stem cells and role of their environment. *Rev. Neurosci.* 30, 45–66.
- Chapouton, P., Skupien, P., Hesl, B., Coolen, M., Moore, J.C., Madelaine, R., Kremmer, E., Faus-Kessler, T., Blader, P., Lawson, N.D., Bally-Cuif, L., 2010. Notch Activity Levels Control the Balance between Quiescence and Recruitment of Adult Neural Stem Cells. *J. Neurosci.* 30, 7961–7974. <https://doi.org/10.1523/JNEUROSCI.6170-09.2010>
- Charmandari, E., Tsigos, C., Chrousos, G., 2005. Endocrinology of the stress response 1. *Annu. Rev. Physiol.* 67, 259–284.
- Chaviaras, S., Mak, P., Ralph, D., Krishnan, L., Broadbear, J.H., 2010. Assessing the antidepressant-like effects of carbetocin, an oxytocin agonist, using a modification of the forced swimming test. *Psychopharmacology (Berl.)* 210, 35–43.
- Cherng, B.-W., Islam, T., Torigoe, M., Tsuboi, T., Okamoto, H., 2020. The dorsal lateral habenula-interpeduncular nucleus pathway is essential for left-right-dependent decision making in zebrafish. *Cell Rep.* 32, 108143.
- Chi, L., Fan, B., Feng, D., Chen, Z., Liu, Z., Hui, Y., Xu, X., Ma, L., Fang, Y., Zhang, Q., 2017. The dorsoventral patterning of human forebrain follows an activation/transformation model. *Cereb. Cortex* 27, 2941–2954.
- Chiu, C.N., Prober, D.A., 2013. Regulation of zebrafish sleep and arousal states: current and prospective approaches. *Front. Neural Circuits* 7, 58.
- Chuang, H.-J., Chang, C.-Y., Ho, H.-P., Chou, M.-Y., 2021. Oxytocin Signaling Acts as a Marker for Environmental Stressors in Zebrafish. *Int. J. Mol. Sci.* 22, 7459.
- Clark, K.J., Boczek, N.J., Ekker, S.C., 2011. Stressing zebrafish for behavioral genetics.
- Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R., Shanahan, F., Dinan, T., Cryan, J., 2013. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol. Psychiatry* 18, 666–673.
- Clarke, G., Stilling, R.M., Kennedy, P.J., Stanton, C., Cryan, J.F., Dinan, T.G., 2014. Minireview: gut microbiota: the neglected endocrine organ. *Mol. Endocrinol.* 28, 1221–1238.
- Clarke, R., Valentin, J., 2009. The History of ICRP and the Evolution of its Policies: Invited by the Commission in October 2008. *Ann. ICRP* 39, 75–110.

- Codega, P., Silva-Vargas, V., Paul, A., Maldonado-Soto, A.R., DeLeo, A.M., Pastrana, E., Doetsch, F., 2014. Prospective identification and purification of quiescent adult neural stem cells from their in vivo niche. *Neuron* 82, 545–559.
- Coe, C.L., Kramer, M., Czéh, B., Gould, E., Reeves, A.J., Kirschbaum, C., Fuchs, E., 2003. Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile rhesus monkeys. *Biol. Psychiatry* 54, 1025–1034.
- Cone Jr, C.D., Cone, C.M., 1976. Induction of mitosis in mature neurons in central nervous system by sustained depolarization. *Science* 192, 155–158.
- Constanzo, J., Midavaine, É., Fouquet, J., Lepage, M., Descoteaux, M., Kirby, K., Tremblay, L., Masson-Côté, L., Geha, S., Longpré, J.-M., 2020. Brain irradiation leads to persistent neuroinflammation and long-term neurocognitive dysfunction in a region-specific manner. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 102, 109954.
- Copplestone, D., Hingston, J., Real, A., 2008. The development and purpose of the FREDERICA radiation effects database. *J. Environ. Radioact.* 99, 1456–1463.
- Cosacak, M.I., Papadimitriou, C., Kizil, C., 2015. Regeneration, plasticity, and induced molecular programs in adult zebrafish brain. *BioMed Res. Int.* 2015.
- Cryan, J.F., Dinan, T.G., 2012. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* 13, 701–712.
- Curtis, M.A., Kam, M., Nannmark, U., Anderson, M.F., Axell, M.Z., Wikkelso, C., Holtås, S., van Roon-Mom, W.M., Bjork-Eriksson, T., Nordborg, C., 2007. Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *science* 315, 1243–1249.
- Cvekl, A., Wang, W.-L., 2009. Retinoic acid signaling in mammalian eye development. *Exp. Eye Res.* 89, 280–291.
- Dafny, N., n.d. Overview of the Nervous System (Section 2, Chapter 1) Neuroscience Online: An Electronic Textbook for the Neurosciences | Department of Neurobiology and Anatomy - The University of Texas Medical School at Houston [WWW Document]. URL <https://nba.uth.tmc.edu/neuroscience/m/s2/chapter01.html> (accessed 6.22.22).
- Davis, D.J., Doerr, H.M., Grzelak, A.K., Busi, S.B., Jasarevic, E., Ericsson, A.C., Bryda, E.C., 2016. *Lactobacillus plantarum* attenuates anxiety-related behavior and protects against stress-induced dysbiosis in adult zebrafish. *Sci. Rep.* 6, 1–11.
- de Abreu, M.S., Demin, K.A., Giacomini, A.C., Amstislavskaya, T.G., Strekalova, T., Maslov, G.O., Kositsin, Y., Petersen, E.V., Kalueff, A.V., 2021. Understanding how stress responses and stress-related behaviors have evolved in zebrafish and mammals. *Neurobiol. Stress* 15, 100405.
- de Andrade Martins, R., n.d. A POOL OF RADIATIONS: BECQUEREL AND POINCARÉ'S CONJECTURE.
- De Castro, F., 2019. Cajal and the spanish neurological school: neuroscience would have been a different story without them. *Front. Cell. Neurosci.* 13, 187.
- Del Bene, F., Wehman, A.M., Link, B.A., Baier, H., 2008. Regulation of neurogenesis by interkinetic nuclear migration through an apical-basal notch gradient. *Cell* 134, 1055–1065.
- Delacroix, D., Guerre, J.-P., Leblanc, P., 2006. *Guide Pratique de Radionucléides et Radioprotection.*
- Demin, K.A., Taranov, A.S., Ilyin, N.P., Lakstygala, A.M., Volgin, A.D., de Abreu, M.S., Strekalova, T., Kalueff, A.V., 2021. Understanding neurobehavioral effects of acute and chronic stress in zebrafish. *Stress* 24, 1–18.

- Demirci, Y., Cucun, G., Poyraz, Y.K., Mohammed, S., Heger, G., Papatheodorou, I., Ozhan, G., 2020. Comparative transcriptome analysis of the regenerating zebrafish telencephalon unravels a resource with key pathways during two early stages and activation of wnt/ β -catenin signaling at the early wound healing stage. *Front. Cell Dev. Biol.* 8, 584604.
- Desouky, O., Ding, N., Zhou, G., 2015. Targeted and non-targeted effects of ionizing radiation. *J. Radiat. Res. Appl. Sci.* 8, 247–254.
- Devine, C., Key, B., 2008. Robo–Slit interactions regulate longitudinal axon pathfinding in the embryonic vertebrate brain. *Dev. Biol.* 313, 371–383.
- Di Giaimo, R., Durovic, T., Barquin, P., Kociaj, A., Lepko, T., Aschenbroich, S., Breunig, C.T., Irmeler, M., Cernilogar, F.M., Schotta, G., 2018. The aryl hydrocarbon receptor pathway defines the time frame for restorative neurogenesis. *Cell Rep.* 25, 3241–3251.
- Diotel, N., Lübke, L., Strähle, U., Rastegar, S., 2020. Common and distinct features of adult neurogenesis and regeneration in the telencephalon of zebrafish and mammals. *Front. Neurosci.* 957.
- Diotel, N., Vaillant, C., Kah, O., Pellegrini, E., 2016. Mapping of brain lipid binding protein (Blbp) in the brain of adult zebrafish, co-expression with aromatase B and links with proliferation. *Gene Expr. Patterns* 20, 42–54.
- Dölen, G., Darvishzadeh, A., Huang, K.W., Malenka, R.C., 2013. Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. *Nature* 501, 179–184.
- Dolezal, A.G., Toth, A.L., 2014. Honey bee sociogenomics: a genome-scale perspective on bee social behavior and health. *Apidologie* 45, 375–395.
- Dong, T., Zhi, L., Bhayana, B., Wu, M.X., 2016. Cortisol-induced immune suppression by a blockade of lymphocyte egress in traumatic brain injury. *J. Neuroinflammation* 13, 1–13.
- Dufourcq-Sekatcheff, E., Cuiné, S., Li-Beisson, Y., Quevarec, L., Richaud, M., Galas, S., Frelon, S., 2021. Deciphering Differential Life Stage Radioinduced Reproductive Decline in *Caenorhabditis elegans* through Lipid Analysis. *Int. J. Mol. Sci.* 22. <https://doi.org/10.3390/ijms221910277>
- Dunn, K., Yoshimaru, H., Otake, M., ANNEGERS, J.F., Schull, W.J., 1990. Prenatal exposure to ionizing radiation and subsequent development of seizures. *Am. J. Epidemiol.* 131, 114–123.
- Durinck, S., Spellman, P.T., Birney, E., Huber, W., 2009. Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. *Nat. Protoc.* 4, 1184–1191.
- Eachus, H., Choi, M.-K., Ryu, S., 2021. The effects of early life stress on the brain and behaviour: Insights from zebrafish models. *Front. Cell Dev. Biol.* 1209.
- Editors of Encyclopaedia Britannica, n.d. midbrain | Anatomy & Function | Britannica [WWW Document]. URL <https://www.britannica.com/science/midbrain> (accessed 6.22.22).
- Ekström, P., Johnsson, C., Ohlin, L., 2001. Ventricular proliferation zones in the brain of an adult teleost fish and their relation to neuromeres and migration (secondary matrix) zones. *J. Comp. Neurol.* 436, 92–110.
- Engeszer, R.E., Da Barbiano, L.A., Ryan, M.J., Parichy, D.M., 2007. Timing and plasticity of shoaling behaviour in the zebrafish, *Danio rerio*. *Anim. Behav.* 74, 1269–1275.

- Erdeljan, P., MacDonald, J., Matthews, S., 2001. Glucocorticoids and serotonin alter glucocorticoid receptor (GR) but not mineralocorticoid receptor (MR) mRNA levels in fetal mouse hippocampal neurons, *in vitro*. *Brain Res.* 896, 130–136.
- Evans, P., Jonkers, G., Steffan, E.-M., Campbell, J., Lloret, C., 2016. Guidelines for the Management of Naturally Occurring Radioactive Material (NORM) in the Oil and Gas Industry. Presented at the SPE International Conference and Exhibition on Health, Safety, Security, Environment, and Social Responsibility, Society of Petroleum Engineers.
- Farin, A., Liu, C.Y., Langmoen, I.A., Apuzzo, M.L., 2009. The biological restoration of central nervous system architecture and function: part 2—emergence of the realization of adult neurogenesis. *Neurosurgery* 64, 581–601.
- Favier, A., 2003. Le stress oxydant. *L'actualité Chim.* 108, 863–832.
- Fernandes, A.M., Fero, K., Arrenberg, A.B., Bergeron, S.A., Driever, W., Burgess, H.A., 2012. Deep brain photoreceptors control light-seeking behavior in zebrafish larvae. *Curr. Biol.* 22, 2042–2047.
- Fernández, I., Gisbert, E., 2011. The effect of vitamin A on flatfish development and skeletogenesis: a review. *Aquaculture* 315, 34–48.
- Ferran, J.L., Hidalgo-Sánchez, M., Puelles, E., 2022. In the footsteps of the prosomeric model. *Front. Neuroanat.* 77.
- Flanagan, J.G., 2006. Neural map specification by gradients. *Curr. Opin. Neurobiol.* 16, 59–66.
- Folgueira, M., Anadón, R., Yáñez, J., 2004. Experimental study of the connections of the telencephalon in the rainbow trout (*Oncorhynchus mykiss*). II: Dorsal area and preoptic region. *J. Comp. Neurol.* 480, 204–233.
- Folley, J.H., Borges, W., Yamawaki, T., 1952. Incidence of leukemia in survivors of the atomic bomb in Hiroshima and Nagasaki, Japan. *Am. J. Med.* 13, 311–321.
- Forlano, P.M., Deitcher, D.L., Bass, A.H., 2005. Distribution of estrogen receptor alpha mRNA in the brain and inner ear of a vocal fish with comparisons to sites of aromatase expression. *J. Comp. Neurol.* 483, 91–113.
- Forlano, P.M., Marchaterre, M., Deitcher, D.L., Bass, A.H., 2010. Distribution of androgen receptor mRNA expression in vocal, auditory, and neuroendocrine circuits in a teleost fish. *J. Comp. Neurol.* 518, 493–512.
- Fu, H., Han, G., Li, H., Liang, X., Hu, D., Zhang, L., Tang, P., 2019. Identification of key genes and pathways involved in the heterogeneity of intrinsic growth ability between neurons after spinal cord injury in adult zebrafish. *Neurochem. Res.* 44, 2057–2067.
- Gamache, G.L., Levinson, D.M., Reeves, D.L., Bidyuk, P.I., Brantley, K.K., 2005. Longitudinal neurocognitive assessments of Ukrainians exposed to ionizing radiation after the Chernobyl nuclear accident. *Arch. Clin. Neuropsychol.* 20, 81–93.
- Ganz, J., Brand, M., 2016. Adult Neurogenesis in Fish. *Cold Spring Harb. Perspect. Biol.* 8. <https://doi.org/10.1101/cshperspect.a019018>
- Ganz, J., Kaslin, J., Hochmann, S., Freudenreich, D., Brand, M., 2010. Heterogeneity and Fgf dependence of adult neural progenitors in the zebrafish telencephalon. *Glia* 58, 1345–1363. <https://doi.org/10.1002/glia.21012>
- Garnier-Laplace, J., 2013. Geras' kin S., Della-Vedova C. et al. Are radiosensitivity data derived from natural field conditions consistent with data from controlled exposures? A case study of Chernobyl wildlife chronically exposed to low dose rates. *J. Env. Radioact* 121, 12.

- Garnier-Laplace, J., Beaugelin-Seiller, K., Hinton, T.G., 2011. Fukushima wildlife dose reconstruction signals ecological consequences.
- Garnier-Laplace, J., Della-Vedova, C., Andersson, P., Copplestone, D., Cailes, C., Beresford, N., Howard, B., Howe, P., Whitehouse, P., 2010. A multi-criteria weight of evidence approach for deriving ecological benchmarks for radioactive substances. *J. Radiol. Prot.* 30, 215.
- Geldmacher-Voss, B., Reugels, A.M., Pauls, S., Campos-Ortega, J.A., 2003. A 90 rotation of the mitotic spindle changes the orientation of mitoses of zebrafish neuroepithelial cells.
- Gemberling, M., Bailey, T.J., Hyde, D.R., Poss, K.D., 2013. The zebrafish as a model for complex tissue regeneration. *Trends Genet.* 29, 611–620.
- Gemmer, A., Mirkes, K., Anneser, L., Eilers, T., Kibat, C., Mathuru, A., Ryu, S., Schuman, E., 2022. Oxytocin receptors influence the development and maintenance of social behavior in zebrafish (*Danio rerio*). *Sci. Rep.* 12, 1–13.
- Genario, R., Giacomini, A.C., Demin, K.A., Dos Santos, B.E., Marchiori, N.I., Volgin, A.D., Bashirzade, A., Amstislavskaya, T.G., de Abreu, M.S., Kalueff, A.V., 2019. The evolutionarily conserved role of melatonin in CNS disorders and behavioral regulation: Translational lessons from zebrafish. *Neurosci. Biobehav. Rev.* 99, 117–127.
- Geng, Y., Peterson, R.T., 2019. The zebrafish subcortical social brain as a model for studying social behavior disorders. *Dis. Model. Mech.* 12, dmm039446.
- Gerlai, R., 2014. Social behavior of zebrafish: from synthetic images to biological mechanisms of shoaling. *J. Neurosci. Methods* 234, 59–65.
- Ghaddar, B., Lübke, L., Couret, D., Rastegar, S., Diotel, N., 2021. Cellular mechanisms participating in brain repair of adult zebrafish and mammals after injury. *Cells* 10, 391.
- Gillies, M., Richardson, D.B., Cardis, E., Daniels, R.D., O'Hagan, J.A., Haylock, R., Laurier, D., Leuraud, K., Moissonnier, M., Schubauer-Berigan, M.K., 2017. Mortality from circulatory diseases and other non-cancer outcomes among nuclear workers in France, the United Kingdom and the United States (INWORKS). *Radiat. Res.* 188, 276–290.
- Goldman, S.A., Windrem, M.S., 2006. Cell replacement therapy in neurological disease. *Philos. Trans. R. Soc. B Biol. Sci.* 361, 1463–1475.
- Goldshmit, Y., Sztal, T.E., Jusuf, P.R., Hall, T.E., Nguyen-Chi, M., Currie, P.D., 2012. Fgf-dependent glial cell bridges facilitate spinal cord regeneration in zebrafish. *J. Neurosci.* 32, 7477–7492.
- Götz, M., Huttner, W.B., 2005. The cell biology of neurogenesis. *Nat. Rev. Mol. Cell Biol.* 6, 777–788. <https://doi.org/10.1038/nrm1739>
- Grandel, H., Brand, M., 2013. Comparative aspects of adult neural stem cell activity in vertebrates. *Dev. Genes Evol.* 223, 131–147.
- Grandel, H., Kaslin, J., Ganz, J., Wenzel, I., Brand, M., 2006. Neural stem cells and neurogenesis in the adult zebrafish brain: origin, proliferation dynamics, migration and cell fate. *Dev. Biol.* 295, 263–277.
- Gregson, J., De Perera, T.B., 2007. Shoaling in eyed and blind morphs of the characin *Astyanax fasciatus* under light and dark conditions. *J. Fish Biol.* 70.
- Griffiths, B.B., Schoonheim, P.J., Ziv, L., Voelker, L., Baier, H., Gahtan, E., 2012. A zebrafish model of glucocorticoid resistance shows serotonergic modulation of the stress response. *Front. Behav. Neurosci.* 6, 68.

- Guédon, R., Maremonti, E., Armant, O., Galas, S., Brede, D.A., Lecomte-Pradines, C., 2021. A systems biology analysis of reproductive toxicity effects induced by multigenerational exposure to ionizing radiation in *C. elegans*. *Ecotoxicol. Environ. Saf.* 225, 112793. <https://doi.org/10.1016/j.ecoenv.2021.112793>
- Guinn, V. Radioactivity. *Encyclopedia of Physical Science and Technology (Third Edition)*, 2003
- Hall, P., Adami, H.-O., Trichopoulos, D., Pedersen, N.L., Lagiou, P., Ekbom, A., Ingvar, M., Lundell, M., Granath, F., 2004. Effect of low doses of ionising radiation in infancy on cognitive function in adulthood: Swedish population based cohort study. *BMJ* 328, 19.
- Harry, G.J., Kraft, A.D., 2012. Microglia in the developing brain: a potential target with lifetime effects. *Neurotoxicology* 33, 191–206.
- Hayama, S., Tsuchiya, M., Ochiai, K., Nakiri, S., Nakanishi, S., Ishii, N., Kato, T., Tanaka, A., Konno, F., Kawamoto, Y., Omi, T., 2017. Small head size and delayed body weight growth in wild Japanese monkey fetuses after the Fukushima Daiichi nuclear disaster. *Sci. Rep.* 7, 3528. <https://doi.org/10.1038/s41598-017-03866-8>
- Haynes, S.E., Hollopeter, G., Yang, G., Kurpius, D., Dailey, M.E., Gan, W.-B., Julius, D., 2006. The P2Y₁₂ receptor regulates microglial activation by extracellular nucleotides. *Nat. Neurosci.* 9, 1512–1519.
- Hellström, N.A., Björk-Eriksson, T., Blomgren, K., Kuhn, H.G., 2009. Differential recovery of neural stem cells in the subventricular zone and dentate gyrus after ionizing radiation. *Stem Cells* 27, 634–641.
- Herzog, C., Pons Garcia, L., Keatinge, M., Greenald, D., Moritz, C., Peri, F., Herrgen, L., 2019. Rapid clearance of cellular debris by microglia limits secondary neuronal cell death after brain injury in vivo. *Development* 146, dev174698.
- Hinz, R.C., de Polavieja, G.G., 2017. Ontogeny of collective behavior reveals a simple attraction rule. *Proc. Natl. Acad. Sci.* 114, 2295–2300.
- Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., Muffato, M., Collins, J.E., Humphray, S., McLaren, K., Matthews, L., 2013. The zebrafish reference genome sequence and its relationship to the human genome. *Nature* 496, 498–503.
- Hui, S.P., Sengupta, D., Lee, S.G.P., Sen, T., Kundu, S., Mathavan, S., Ghosh, S., 2014. Genome wide expression profiling during spinal cord regeneration identifies comprehensive cellular responses in zebrafish. *PloS One* 9, e84212.
- Huising, M., Van Schooten, C., Taverne-Thiele, A., Hermsen, T., Verburg-van Kemenade, B., Flik, G., 2004. Structural characterisation of a cyprinid (*Cyprinus carpio* L.) CRH, CRH-BP and CRH-R1, and the role of these proteins in the acute stress response. *J. Mol. Endocrinol.* 32, 627–648.
- Hurem, S., Martín, L.M., Brede, D.A., Skjerve, E., Nourizadeh-Lillabadi, R., Lind, O.C., Christensen, T., Berg, V., Teien, H.-C., Salbu, B., 2017. Dose-dependent effects of gamma radiation on the early zebrafish development and gene expression. *PloS One* 12, e0179259.
- ICRP, 2008. Publication 108. Environmental Protection: the Concept and Use of Reference Animals and Plants, *Annals of the ICRP*.
- ICRP, 1991. Publication 60. Recommendations of the International Commission on Radiological Protection.
- ICRP, 1977. Publication 26. Recommendations of the International Commission on Radiological Protection.

- Igarashi, K., Iwai, H., Tanaka, K., Kuwahara, Y., Kitanaka, J., Kitanaka, N., Kurimasa, A., Tomita, K., Sato, T., 2022. Neuroprotective effect of oxytocin on cognitive dysfunction, DNA damage, and intracellular chloride disturbance in young mice after cranial irradiation. *Biochem. Biophys. Res. Commun.* 612, 1–7.
- IRSN, 2013. *Histoires de radioactivité Prudence : Histoire de la radioprotection.*
- Iwasaki, K., Taguchi, M., Bonkowsky, J.L., Kuwada, J.Y., 2013. Expression of arginine vasotocin receptors in the developing zebrafish CNS. *Gene Expr. Patterns* 13, 335–342.
- Jaylet, T., Quintens, R., Benotmane, M.A., Luukkonen, J., Tanaka III, I.B., Ibanez, C., Durand, C., Sachana, M., Azimzadeh, O., Adam-Guillermin, C., 2022. Development of an adverse outcome pathway for radiation-induced microcephaly via expert consultation and machine learning. *Int. J. Radiat. Biol.* 1–11.
- Jesuthasan, S., 2012. Fear, anxiety, and control in the zebrafish. *Dev. Neurobiol.* 72, 395–403.
- Ju Shin, Y., Kyun Park, S., Jung Jung, Y., Na Kim, Y., Sung Kim, K., Kyu Park, O., Kwon, S.-H., Ho Jeon, S., Trinh, L.A., Fraser, S.E., 2015. Nanobody-targeted E3-ubiquitin ligase complex degrades nuclear proteins. *Sci. Rep.* 5, 1–11.
- Jurek, B., Slattery, D.A., Hiraoka, Y., Liu, Y., Nishimori, K., Aguilera, G., Neumann, I.D., Van Den Burg, E.H., 2015. Oxytocin regulates stress-induced Crf gene transcription through CREB-regulated transcription coactivator 3. *J. Neurosci.* 35, 12248–12260.
- Kalueff, A.V., Stewart, A.M., Gerlai, R., 2014. Zebrafish as an emerging model for studying complex brain disorders. *Trends Pharmacol. Sci.* 35, 63–75.
- Kamstra, J.H., Hurem, S., Martin, L.M., Lindeman, L.C., Legler, J., Oughton, D., Salbu, B., Brede, D.A., Lyche, J.L., Aleström, P., 2018. Ionizing radiation induces transgenerational effects of DNA methylation in zebrafish. *Sci. Rep.* 8, 1–13.
- Kanagaraj, P., Chen, J.Y., Skaggs, K., Qadeer, Y., Conner, M., Cutler, N., Richmond, J., Kommidi, V., Poles, A., Affrunti, D., 2020. Microglia stimulate zebrafish brain repair via a specific inflammatory cascade. *BioRxiv.*
- Kaplan, M.S., Hinds, J.W., 1977. Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. *Science* 197, 1092–1094.
- Kaya, H., Delibas, N., Serteser, M., Ulukaya, E., Özkaya, O., 1999. The effect of melatonin on lipid peroxidation during radiotherapy in female rats. *Strahlenther. Onkol.* 175, 285–288.
- Kempf, S.J., Casciati, A., Buratovic, S., Janik, D., Toerne, C. von, Ueffing, M., Neff, F., Moertl, S., Stenerlöw, B., Saran, A., Atkinson, M.J., Eriksson, P., Pazzaglia, S., Tapio, S., 2014. The cognitive defects of neonatally irradiated mice are accompanied by changed synaptic plasticity, adult neurogenesis and neuroinflammation. *Mol. Neurodegener.* 9, 57. <https://doi.org/10.1186/1750-1326-9-57>
- Kim, H.-K., Lee, D., Kim, E., Jeong, I., Kim, S., Kim, B.-J., Park, H.-C., 2020. Notch signaling controls oligodendrocyte regeneration in the injured telencephalon of adult zebrafish. *Exp. Neurobiol.* 29, 417.
- Kishimoto, N., Alfaro-Cervello, C., Shimizu, K., Asakawa, K., Urasaki, A., Nonaka, S., Kawakami, K., Garcia-Verdugo, J.M., Sawamoto, K., 2011. Migration of neuronal precursors from the telencephalic ventricular zone into the olfactory bulb in adult zebrafish. *J. Comp. Neurol.* 519, 3549–3565.
- Kishimoto, N., Shimizu, K., Sawamoto, K., 2012. Neuronal regeneration in a zebrafish model of adult brain injury. *Dis. Model. Mech.* 5, 200–209.

- Kizil, C., Dudczig, S., Kyritsis, N., Machate, A., Blaesche, J., Kroehne, V., Brand, M., 2012a. The chemokine receptor *cxcr5* regulates the regenerative neurogenesis response in the adult zebrafish brain. *Neural Develop.* 7, 27. <https://doi.org/10.1186/1749-8104-7-27>
- Kizil, C., Kaslin, J., Kroehne, V., Brand, M., 2012b. Adult neurogenesis and brain regeneration in zebrafish. *Dev. Neurobiol.* 72, 429–461.
- Kizil, C., Kyritsis, N., Dudczig, S., Kroehne, V., Freudenreich, D., Kaslin, J., Brand, M., 2012c. Regenerative neurogenesis from neural progenitor cells requires injury-induced expression of *Gata3*.
- Klaassen, T., Riedel, W.J., van Praag, H.M., Menheere, P.P., Griez, E., 2002. Neuroendocrine response to meta-chlorophenylpiperazine and ipsapirone in relation to anxiety and aggression. *Psychiatry Res.* 113, 29–40.
- Kopp, C., Vogel, E., Rettori, M.-C., Delagrang, P., Guardiola-Lemaître, B., Misslin, R., 1999. Effects of melatonin on neophobic responses in different strains of mice. *Pharmacol. Biochem. Behav.* 63, 521–526.
- Krammer, S., Kleim, B., Simmen-Janevska, K., Maercker, A., 2016. Childhood trauma and complex posttraumatic stress disorder symptoms in older adults: A study of direct effects and social-interpersonal factors as potential mediators. *J. Trauma Dissociation* 17, 593–607.
- Kriegstein, A., Alvarez-Buylla, A., 2009. The glial nature of embryonic and adult neural stem cells. *Annu. Rev. Neurosci.* 32, 149–184. <https://doi.org/10.1146/annurev.neuro.051508.135600>
- Kroehne, V., Freudenreich, D., Hans, S., Kaslin, J., Brand, M., 2011. Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors. *Development* 138, 4831–4841.
- Kumamoto, T., Hanashima, C., 2014. Neuronal subtype specification in establishing mammalian neocortical circuits. *Neurosci. Res.* 86, 37–49.
- Kuzmic, M., 2018. Role of protein and DNA damage in biological response to radiation and aging.
- Kyritsis, N., Kizil, C., Zocher, S., Kroehne, V., Kaslin, J., Freudenreich, D., Iltzsche, A., Brand, M., 2012. Acute inflammation initiates the regenerative response in the adult zebrafish brain. *Science* 338, 1353–1356.
- Labusch, M., Mancini, L., Morizet, D., Bally-Cuif, L., 2020. Conserved and divergent features of adult neurogenesis in zebrafish. *Front. Cell Dev. Biol.* 8, 525.
- Lamb, T., Pugh Jr, E.N., 2004. Dark adaptation and the retinoid cycle of vision. *Prog. Retin. Eye Res.* 23, 307–380.
- Landin, J., Hovey, D., Xu, B., Lagman, D., Zettergren, A., Larhammar, D., Kettunen, P., Westberg, L., 2020. Oxytocin receptors regulate social preference in zebrafish. *Sci. Rep.* 10, 1–12.
- Lanfume, L., Mongeau, R., Cohen-Salmon, C., Hamon, M., 2008. Corticosteroid–serotonin interactions in the neurobiological mechanisms of stress-related disorders. *Neurosci. Biobehav. Rev.* 32, 1174–1184.
- Larson, E.T., O'Malley, D.M., Melloni Jr, R.H., 2006. Aggression and vasotocin are associated with dominant–subordinate relationships in zebrafish. *Behav. Brain Res.* 167, 94–102.
- Lauritzen, I., De Weille, J.R., Lazdunski, M., 1997. The potassium channel opener (–)-cromakalim prevents glutamate-induced cell death in hippocampal neurons. *J. Neurochem.* 69, 1570–1579.

- Lauritzen, I., Zanzouri, M., Honoré, E., Duprat, F., Ehrengruber, M.U., Lazdunski, M., Patel, A.J., 2003. K⁺-dependent cerebellar granule neuron apoptosis: role of TASK leak K⁺ channels. *J. Biol. Chem.* 278, 32068–32076.
- Lazarini, F., Mouthon, M.-A., Gheusi, G., de Chaumont, F., Olivo-Marin, J.-C., Lamarque, S., Abrous, D.N., Boussin, F.D., Lledo, P.-M., 2009. Cellular and behavioral effects of cranial irradiation of the subventricular zone in adult mice. *PLoS One* 4, e7017–e7017. <https://doi.org/10.1371/journal.pone.0007017>
- Lee, J.-G., Cho, H.-J., Jeong, Y.-M., Lee, J.-S., 2021. Genetic approaches using zebrafish to study the microbiota–gut–brain axis in neurological disorders. *Cells* 10, 566.
- Lerebours, A., Gudkov, D., Nagorskaya, L., Kaglyan, A., Rizewski, V., Leshchenko, A., Bailey, E.H., Bakir, A., Ovsyanikova, S., Laptev, G., 2018. Impact of environmental radiation on the health and reproductive status of fish from Chernobyl. *Environ. Sci. Technol.* 52, 9442–9450.
- Levin, M., 2009. Bioelectric mechanisms in regeneration: unique aspects and future perspectives. Presented at the Seminars in cell & developmental biology, Elsevier, pp. 543–556.
- Li, R., Liao, G., Yin, G., Wang, B., Yan, M., Lin, X., Zhang, W., Chen, X., Du, S., Yuan, Y., 2018. Ionizing Radiation Blocks Hair Cell Regeneration in Zebrafish Lateral Line Neuromasts by Preventing Wnt Signaling. *Mol. Neurobiol.* 55, 1639–1651. <https://doi.org/10.1007/s12035-017-0430-9>
- Li, X., Montgomery, J., Cheng, W., Noh, J.H., Hyde, D.R., Li, L., 2012. Pineal photoreceptor cells are required for maintaining the circadian rhythms of behavioral visual sensitivity in zebrafish. *PLoS One* 7, e40508.
- Lim, F.T., Ogawa, S., Parhar, I.S., 2016. Spred-2 expression is associated with neural repair of injured adult zebrafish brain. *J. Chem. Neuroanat.* 77, 176–186.
- Lima-Cabello, E., Díaz-Casado, M.E., Guerrero, J.A., Ojalora, B.B., Escames, G., López, L.C., Reiter, R.J., Acuña-Castroviejo, D., 2014. A review of the melatonin functions in zebrafish physiology. *J. Pineal Res.* 57, 1–9. <https://doi.org/10.1111/jpi.12149>
- Lindeyer, C.M., Langen, E.M., Swaney, W.T., Reader, S.M., 2015. Nonapeptide influences on social behaviour: effects of vasotocin and isotocin on shoaling and interaction in zebrafish. *Behaviour* 152, 897–915.
- Lindsey, B.W., Hall, Z.J., Heuzé, A., Joly, J.-S., Tropepe, V., Kaslin, J., 2018. The role of neuro-epithelial-like and radial-glia stem and progenitor cells in development, plasticity, and repair. *Prog. Neurobiol.* 170, 99–114.
- Liu, X., Zhou, Y., Wang, S., Guan, H., Hu, S., Huang, R., Zhou, P., 2019. Impact of low-dose ionizing radiation on the composition of the gut microbiota of mice. *Toxicol. Sci.* 171, 258–268.
- Llorens-Bobadilla, E., Zhao, S., Baser, A., Saiz-Castro, G., Zwadlo, K., Martin-Villalba, A., 2015. Single-cell transcriptomics reveals a population of dormant neural stem cells that become activated upon brain injury. *Cell Stem Cell* 17, 329–340.
- Lowe, X.R., Bhattacharya, S., Marchetti, F., Wyrobek, A.J., 2009. Early Brain Response to Low-Dose Radiation Exposure Involves Molecular Networks and Pathways Associated with Cognitive Functions, Advanced Aging and Alzheimer’s Disease. *Radiat. Res.* 171, 53–65. <https://doi.org/10.1667/RR1389.1>
- Ludwig, M., 1998. Dendritic release of vasopressin and oxytocin. *J. Neuroendocrinol.* 10, 881–895.
- Luo, L., Song, S., Ezenwukwa, C.C., Jalali, S., Sun, B., Sun, D., 2021. Ion channels and transporters in microglial function in physiology and brain diseases. *Neurochem. Int.* 142, 104925.

- Malley, M.C., 2011. Radioactivity: a history of a mysterious science. OUP USA.
- Manuel, R., Iglesias Gonzalez, A.B., Habicher, J., Koning, H.K., Boije, H., 2021. Characterization of Individual Projections Reveal That Neuromasts of the Zebrafish Lateral Line are Innervated by Multiple Inhibitory Efferent Cells. *Front. Neuroanat.* 15, 666109.
- März, M., Chapouton, P., Diotel, N., Vaillant, C., Hesl, B., Takamiya, M., Lam, C.S., Kah, O., Bally-Cuif, L., Strähle, U., 2010. Heterogeneity in progenitor cell subtypes in the ventricular zone of the zebrafish adult telencephalon. *Glia* 58, 870–888. <https://doi.org/10.1002/glia.20971>
- März, M., Schmidt, R., Rastegar, S., Strähle, U., 2011. Regenerative response following stab injury in the adult zebrafish telencephalon. *Dev. Dyn.* 240, 2221–2231.
- Mathuru, A., Jesuthasan, S., 2013. The medial habenula as a regulator of anxiety in adult zebrafish.
- McAleer, M.F., Davidson, C., Davidson, W.R., Yentzer, B., Farber, S.A., Rodeck, U., Dicker, A.P., 2005. Novel use of zebrafish as a vertebrate model to screen radiation protectors and sensitizers. *Int. J. Radiat. Oncol. Biol. Phys.* 61, 10–13.
- McMillan, K.A., Sareen, J., Asmundson, G.J., 2014. Social anxiety disorder is associated with PTSD symptom presentation: an exploratory study within a nationally representative sample. *J. Trauma. Stress* 27, 602–609.
- Meloni, E.G., Reedy, C.L., Cohen, B.M., Carlezon Jr, W.A., 2008. Activation of raphe efferents to the medial prefrontal cortex by corticotropin-releasing factor: correlation with anxiety-like behavior. *Biol. Psychiatry* 63, 832–839.
- Meltz, M.L., Reiter, R.J., Herman, T.S., 1999. Melatonin and protection from genetic damage in blood and bone marrow: Whole-body irradiation studies in mice. *J. Pineal Res.* 27, 221–225.
- Meyer, M.A., Anstötz, M., Ren, L.Y., Fiske, M.P., Guedea, A.L., Grayson, V.S., Schroth, S.L., Cicvaric, A., Nishimori, K., Maccaferri, G., 2020. Stress-related memories disrupt sociability and associated patterning of hippocampal activity: a role of hilar oxytocin receptor-positive interneurons. *Transl. Psychiatry* 10, 1–14.
- Michaud, J.L., DeRossi, C., May, N.R., Holdener, B.C., Fan, C.-M., 2000. ARNT2 acts as the dimerization partner of SIM1 for the development of the hypothalamus. *Mech. Dev.* 90, 253–261.
- Michaud, J.L., Rosenquist, T., May, N.R., Fan, C.-M., 1998. Development of neuroendocrine lineages requires the bHLH–PAS transcription factor SIM1. *Genes Dev.* 12, 3264–3275.
- Moe, M.C., Varghese, M., Danilov, A.I., Westerlund, U., Ramm-Petersen, J., Brundin, L., Svensson, M., Berg-Johnsen, J., Langmoen, I.A., 2005. Multipotent progenitor cells from the adult human brain: neurophysiological differentiation to mature neurons. *Brain* 128, 2189–2199.
- Møller, A.P., Bonisoli-Alquati, A., Rudolfson, G., Mousseau, T.A., 2011. Chernobyl Birds Have Smaller Brains. *PLOS ONE* 6, e16862. <https://doi.org/10.1371/journal.pone.0016862>
- Mueller, K.P., Neuhaus, S.C., 2010. Quantitative measurements of the optokinetic response in adult fish. *J. Neurosci. Methods* 186, 29–34.
- Murat El Houdigui, S., Adam-Guillermin, C., Armant, O., 2020. Ionising Radiation Induces Promoter DNA Hypomethylation and Perturbs Transcriptional Activity of Genes Involved in Morphogenesis during Gastrulation in Zebrafish. *Int. J. Mol. Sci.* 21, 4014.

- Murat El Houdigui, S., Adam-Guillermin, C., Loro, G., Arcanjo, C., Frelon, S., Floriani, M., Dubourg, N., Baudalet, E., Audebert, S., Camoin, L., 2019. A systems biology approach reveals neuronal and muscle developmental defects after chronic exposure to ionising radiation in zebrafish. *Sci. Rep.* 9, 1–15.
- Neumann, I.D., Landgraf, R., 2012. Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. *Trends Neurosci.* 35, 649–659.
- Newman, S.W., 1999. The medial extended amygdala in male reproductive behavior a node in the mammalian social behavior network. *Ann. N. Y. Acad. Sci.* 877, 242–257.
- Nuckels, R.J., Ng, A., Darland, T., Gross, J.M., 2009. The vacuolar-ATPase complex regulates retinoblast proliferation and survival, photoreceptor morphogenesis, and pigmentation in the zebrafish eye. *Invest. Ophthalmol. Vis. Sci.* 50, 893–905.
- O’Connell, L.A., Hofmann, H.A., 2011. The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. *J. Comp. Neurol.* 519, 3599–3639.
- Ogawa, S., Parhar, I.S., 2022. Role of Habenula in Social and Reproductive Behaviors in Fish: Comparison With Mammals. *Front. Behav. Neurosci.* 15.
- Okamoto, H., Agetsuma, M., Aizawa, H., 2012. Genetic dissection of the zebrafish habenula, a possible switching board for selection of behavioral strategy to cope with fear and anxiety. *Dev. Neurobiol.* 72, 386–394.
- Okamoto, H., Cherng, B.-W., Nakajo, H., Chou, M.-Y., Kinoshita, M., 2021. Habenula as the experience-dependent controlling switchboard of behavior and attention in social conflict and learning. *Curr. Opin. Neurobiol.* 68, 36–43.
- Oliveira, R.F., 2013. Mind the fish: zebrafish as a model in cognitive social neuroscience. *Front. Neural Circuits* 0. <https://doi.org/10.3389/fncir.2013.00131>
- Onaka, T., Takayanagi, Y., Yoshida, M., 2012. Roles of oxytocin neurones in the control of stress, energy metabolism, and social behaviour. *J. Neuroendocrinol.* 24, 587–598.
- Osman, A.M., Sun, Y., Burns, T.C., He, L., Kee, N., Oliva-Vilarnau, N., Alevyzaki, A., Zhou, K., Louhivuori, L., Uhlén, P., 2020. Radiation triggers a dynamic sequence of transient microglial alterations in juvenile brain. *Cell Rep.* 31, 107699.
- O’Sullivan, E., Barrett, E., Grenham, S., Fitzgerald, P., Stanton, C., Ross, R., Quigley, E., Cryan, J., Dinan, T., 2011. BDNF expression in the hippocampus of maternally separated rats: does *Bifidobacterium breve* 6330 alter BDNF levels? *Benef. Microbes* 2, 199–207.
- Otake, M., Schull, W.J., 1998. Radiation-related brain damage and growth retardation among the prenatally exposed atomic bomb survivors. *Int. J. Radiat. Biol.* 74, 159–171.
- Palczewski, K., 2006. G protein-coupled receptor rhodopsin. *Annu Rev Biochem* 75, 743–767.
- Park, S.H., Kang, J.O., 2011. Basics of particle therapy I: physics. *Radiat. Oncol. J.* 29, 135.
- Partridge, B.L., Pitcher, T.J., 1980. The sensory basis of fish schools: Relative roles of lateral line and vision. *J. Comp. Physiol.* 135, 315–325. <https://doi.org/10.1007/BF00657647>
- Pedersen, C.A., Prange Jr, A.J., 1979. Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin. *Proc. Natl. Acad. Sci.* 76, 6661–6665.
- Pentreath, R., 1998. Radiological protection criteria for the natural environment. *Radiat. Prot. Dosimetry* 75, 175–179.
- Plummer, G., 1952. Anomalies occurring in children exposed in utero to the atomic bomb in Hiroshima. *Pediatrics* 10, 687–693.

- Prabhudesai, S.N., Cameron, D.A., Stenkamp, D.L., 2005. Targeted effects of retinoic acid signaling upon photoreceptor development in zebrafish. *Dev. Biol.* 287, 157–167.
- Prigent, A., 2012. Marie Curie à l'origine de la radiothérapie.
- Purves, D., Augustine, G., Fitzpatrick, D., Katz, L., LaMantia, A., McNamara, J., Williams, S., 2001. The initial formation of the nervous system: gastrulation and neurulation. *Neuroscience 2nd Ed Sunderland MA Sinauer Assoc.* 520–535.
- Quintens, R., Verreert, T., Janssen, A., Neefs, M., Leysen, L., Michaux, A., Verslegers, M., Samari, N., Pani, G., Verheyde, J., 2015. Identification of novel radiation-induced p53-dependent transcripts extensively regulated during mouse brain development. *Biol. Open* 4, 331–344.
- Raber, J., Rola, R., LeFevour, A., Morhardt, D., Curley, J., Mizumatsu, S., VandenBerg, S.R., Fike, J.R., 2004. Radiation-induced cognitive impairments are associated with changes in indicators of hippocampal neurogenesis. *Radiat. Res.* 162, 39–47.
- Raymond, P.A., Barthel, L.K., Bernardos, R.L., Perkowski, J.J., 2006. Molecular characterization of retinal stem cells and their niches in adult zebrafish. *BMC Dev. Biol.* 6, 1–17.
- Reisz, J.A., Bansal, N., Qian, J., Zhao, W., Furdul, C.M., 2014. Effects of ionizing radiation on biological molecules—mechanisms of damage and emerging methods of detection. *Antioxid. Redox Signal.* 21, 260–292.
- Reynolds, B.A., Weiss, S., 1992. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *science* 255, 1707–1710.
- Ribeiro, D., Nunes, A.R., Glikberg, M., Anbalagan, S., Levkowitz, G., Oliveira, R.F., 2020. Oxytocin receptor signalling modulates novelty recognition but not social preference in zebrafish. *J. Neuroendocrinol.* 32, e12834.
- Roos, M., Schachner, M., Bernhardt, R.R., 1999. Zebrafish semaphorin Z1b inhibits growing motor axons in vivo. *Mech. Dev.* 87, 103–117.
- Roy, L., Gruel, G., Vaurijoux, A., 2009. γ -H2AX as protein bio marker for radiation exposure. *Ann. Ist. Super. Sanita* 45, 272–277.
- Saaltink, D.-J., Vreugdenhil, E., 2014. Stress, glucocorticoid receptors, and adult neurogenesis: a balance between excitation and inhibition? *Cell. Mol. Life Sci.* 71, 2499–2515.
- Sadoul, B., Alfonso, S., Bessa, E., Bouchareb, A., Blondeau-Bidet, E., Clair, P., Chatain, B., Begout, M.-L., Geffroy, B., 2018. Enhanced brain expression of genes related to cell proliferation and neural differentiation is associated with cortisol receptor expression in fishes. *Gen. Comp. Endocrinol.* 267, 76–81.
- Saif, M., Chatterjee, D., Buske, C., Gerlai, R., 2013. Sight of conspecific images induces changes in neurochemistry in zebrafish. *Behav. Brain Res.* 243, 294–299.
- Sanai, N., Nguyen, T., Ihrie, R.A., Mirzadeh, Z., Tsai, H.-H., Wong, M., Gupta, N., Berger, M.S., Huang, E., Garcia-Verdugo, J.-M., 2011. Corridors of migrating neurons in the human brain and their decline during infancy. *Nature* 478, 382–386.
- Sanchez-Gonzalez, R., Koupourtidou, C., Lepko, T., Zambusi, A., Novoselc, K.T., Durovic, T., Aschenbroich, S., Schwarz, V., Breunig, C.T., Straka, H., 2022. Innate Immune Pathways Promote Oligodendrocyte Progenitor Cell Recruitment to the Injury Site in Adult Zebrafish Brain. *Cells* 11, 520.
- Saul, K.E., Koke, J.R., García, D.M., 2010. Activating transcription factor 3 (ATF3) expression in the neural retina and optic nerve of zebrafish during optic nerve regeneration. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 155, 172–182.
- Scerbina, T., Chatterjee, D., Gerlai, R., 2012. Dopamine receptor antagonism disrupts social preference in zebrafish: a strain comparison study. *Amino Acids* 43, 2059–2072.

- Schmidt, R., Beil, T., Strähle, U., Rastegar, S., 2014. Stab wound injury of the zebrafish adult telencephalon: a method to investigate vertebrate brain neurogenesis and regeneration. *JoVE J. Vis. Exp.* e51753.
- Schmidt, R., Strähle, U., Scholpp, S., 2013. Neurogenesis in zebrafish – from embryo to adult. *Neural Develop.* 8, 3. <https://doi.org/10.1186/1749-8104-8-3>
- Schreck, C.B., Tort, L., 2016. The concept of stress in fish, in: *Fish Physiology*. Elsevier, pp. 1–34.
- Schull, W.J., Otake, M., 1999. Cognitive function and prenatal exposure to ionizing radiation. *Teratology* 59, 222–226.
- Shenoy, A., Banerjee, M., Upadhyaya, A., Bagwe-Parab, S., Kaur, G., 2022. The Brilliance of the Zebrafish Model: Perception on Behavior and Alzheimer’s Disease. *Front. Behav. Neurosci.* 16.
- Shimizu, Y., Kiyooka, M., Ohshima, T., 2021. Transcriptome analyses reveal IL6/Stat3 signaling involvement in radial glia proliferation after stab wound injury in the adult Zebrafish optic tectum. *Front. Cell Dev. Biol.* 9, 668408.
- Sieger, D., Moritz, C., Ziegenhals, T., Prykhozhiy, S., Peri, F., 2012. Long-range Ca²⁺ waves transmit brain-damage signals to microglia. *Dev. Cell* 22, 1138–1148.
- Singh, A., Subhashini, N., Sharma, S., Mallick, B., 2013. Involvement of the α 1-adrenoceptor in sleep–waking and sleep loss-induced anxiety behavior in zebrafish. *Neuroscience* 245, 136–147.
- Spence, R., Gerlach, G., Lawrence, C., Smith, C., 2008. The behaviour and ecology of the zebrafish, *Danio rerio*. *Biol. Rev.* 83, 13–34.
- Stillwell, E., Cone, CM, Cone, CD, 1973. Stimulation of DNA synthesis in CNS neurones by sustained depolarisation. *Nature. New Biol.* 246, 110–111.
- Stoehr, J.D., Cramer, C.P., North, W.G., 1992. Oxytocin and vasopressin hexapeptide fragments have opposing influences on conditioned freezing behavior. *Psychoneuroendocrinology* 17, 267–271.
- Su, L.-J., Zhang, J.-H., Gomez, H., Murugan, R., Hong, X., Xu, D., Jiang, F., Peng, Z.-Y., 2019. Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. *Oxid. Med. Cell. Longev.* 2019.
- Sueda, R., Imayoshi, I., Harima, Y., Kageyama, R., 2019. High Hes1 expression and resultant Ascl1 suppression regulate quiescent vs. active neural stem cells in the adult mouse brain. *Genes Dev.* 33, 511–523.
- Takayanagi, Y., Yoshida, M., Bielsky, I.F., Ross, H.E., Kawamata, M., Onaka, T., Yanagisawa, T., Kimura, T., Matzuk, M.M., Young, L.J., 2005. Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. *Proc. Natl. Acad. Sci.* 102, 16096–16101.
- Teles, M.C., Cardoso, S.D., Oliveira, R.F., 2016. Social plasticity relies on different neuroplasticity mechanisms across the brain social decision-making network in zebrafish. *Front. Behav. Neurosci.* 10, 16.
- Teles, M.C., Dahlbom, S.J., Winberg, S., Oliveira, R.F., 2013. Social modulation of brain monoamine levels in zebrafish. *Behav. Brain Res.* 253, 17–24.
- Tessier-Lavigne, M., Goodman, C.S., 1996. The molecular biology of axon guidance. *Science* 274, 1123–1133.
- Than-Trong, E., Ortica-Gatti, S., Mella, S., Nepal, C., Alunni, A., Bally-Cuif, L., 2018. Neural stem cell quiescence and stemness are molecularly distinct outputs of the Notch3 signalling cascade in the vertebrate adult brain.
- Trolese, M.C., Mariani, A., Terao, M., de Paola, M., Fabbrizio, P., Sironi, F., Kurosaki, M., Bonanno, S., Marcuzzo, S., Bernasconi, P., 2020. CXCL13/CXCR5 signalling is pivotal

- to preserve motor neurons in amyotrophic lateral sclerosis. *EBioMedicine* 62, 103097.
- Tsata, V., Wehner, D., 2021. Know How to Regrow—Axon Regeneration in the Zebrafish Spinal Cord. *Cells* 10, 1404.
- Tubiana, M., 1996. Wilhelm Conrad Röntgen and the discovery of X-rays. *Bull. Académie Natl. Médecine* 180, 97–108.
- UNSCEAR, 2010. UNSCEAR. Report of the United Nations Scientific Committee on the Effects of Atomic Radiation 2010: 57th session: summary of low_dose radiation effects on health.
- UNSCEAR, 2008. Sources and effects of ionizing radiation. Report to the general assembly with scientific annexes. .
- Uranium glass fluorescing – acheter une photo – 11725688 | Science Photo Library [WWW Document], n.d. URL <https://www.sciencephoto.fr/image/11725688-Uranium-glass-fluorescing> (accessed 10.18.22).
- Verreet, T., Rangarajan, J.R., Quintens, R., Verslegers, M., Lo, A.C., Govaerts, K., Neefs, M., Leysen, L., Baatout, S., Maes, F., 2016. Persistent impact of in utero irradiation on mouse brain structure and function characterized by MR imaging and behavioral analysis. *Front. Behav. Neurosci.* 10, 83.
- Viales, R.R., Diotel, N., Ferg, M., Armant, O., Eich, J., Alunni, A., März, M., Bally-Cuif, L., Rastegar, S., Strähle, U., 2015. The helix-loop-helix protein id1 controls stem cell proliferation during regenerative neurogenesis in the adult zebrafish telencephalon. *Stem Cells* 33, 892–903.
- Wagle, R., Song, Y.-H., 2020. Ionizing radiation reduces larval brain size by inducing premature differentiation of *Drosophila* neural stem cells. *Biochem. Biophys. Res. Commun.* 523, 555–560.
- Wang, C., Liu, F., Liu, Y.-Y., Zhao, C.-H., You, Y., Wang, L., Zhang, J., Wei, B., Ma, T., Zhang, Q., 2011. Identification and characterization of neuroblasts in the subventricular zone and rostral migratory stream of the adult human brain. *Cell Res.* 21, 1534–1550.
- Wang, L.-F., Huang, S.-B., Zhao, H.-D., Liu, C.-J., Yao, L., Shen, Y.-Q., 2017. Activating transcription factor 3 promotes spinal cord regeneration of adult zebrafish. *Biochem. Biophys. Res. Commun.* 488, 522–527.
- Wang, R.N., Green, J., Wang, Z., Deng, Y., Qiao, M., Peabody, M., Zhang, Q., Ye, J., Yan, Z., Denduluri, S., 2014. Bone Morphogenetic Protein (BMP) signaling in development and human diseases. *Genes Dis.* 1, 87–105.
- Wee, C.L., Nikitchenko, M., Wang, W.-C., Luks-Morgan, S.J., Song, E., Gagnon, J.A., Randlett, O., Bianco, I.H., Lacoste, A., Glushenkova, E., 2019. Zebrafish oxytocin neurons drive nocifensive behavior via brainstem premotor targets. *Nat. Neurosci.* 22, 1477–1492.
- Wendelaar Bonga, S.E., 1997. The stress response in fish. *Physiol. Rev.* 77, 591–625.
- Wetzler, S., Asnis, G.M., DeLecuona, J.M., Kalus, O., 1996. Serotonin function in panic disorder: intravenous administration of meta-chlorophenylpiperazine. *Psychiatry Res.* 64, 77–82.
- Whicker, F., Bedford, J., 1995. Protection of the natural environment from ionizing radiation. Are specific criteria needed? Presented at the Environmental impact of radioactive releases. Proceedings of an international symposium.
- Williams, A., 2014. Central nervous system regeneration—where are we? *QJM Int. J. Med.* 107, 335–339.

- Winberg, M.L., Mitchell, K.J., Goodman, C.S., 1998. Genetic analysis of the mechanisms controlling target selection: complementary and combinatorial functions of netrins, semaphorins, and IgCAMs. *Cell* 93, 581–591.
- Windle, R., Shanks, N., Lightman, S.L., Ingram, C.D., 1997. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. *Endocrinology* 138, 2829–2834.
- Winter, J., Jurek, B., 2019. The interplay between oxytocin and the CRF system: regulation of the stress response. *Cell Tissue Res.* 375, 85–91.
- Wong, E., Herbert, J., 2006. Raised circulating corticosterone inhibits neuronal differentiation of progenitor cells in the adult hippocampus. *Neuroscience* 137, 83–92.
- Wu, Y.S., Chen, S.N., 2014. Apoptotic cell: linkage of inflammation and wound healing.
- Wullimann, M., Rupp, B., Reichert, H., Easter Jr, S.S., 1996. Neuroanatomy of the Zebrafish brain: a topological atlas. *Trends Neurosci.* 19, 525.
- Xu, Q., Alldus, G., Macdonald, R., Wilkinson, D.G., Holder, N., 1996. Function of the Eph-related kinase rtk1 in patterning of the zebrafish forebrain. *Nature* 381, 319–322.
- Yamamoto, N., Ishikawa, Y., Yoshimoto, M., Xue, H.-G., Bahaxar, N., Sawai, N., Yang, C.-Y., Ozawa, H., Ito, H., 2007. A new interpretation on the homology of the teleostean telencephalon based on hodology and a new eversion model. *Brain. Behav. Evol.* 69, 96–104.
- Yamamoto, K., Bloch, S., Vernier, P., New perspective on the regionalization of the anterior forebrain in *Osteichthyes*. *Development, Growth & differentiation.* 59(4), 175–187
- Yano, J.M., Yu, K., Donaldson, G.P., Shastri, G.G., Ann, P., Ma, L., Nagler, C.R., Ismagilov, R.F., Mazmanian, S.K., Hsiao, E.Y., 2015. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* 161, 264–276.
- Yin, E., Nelson, D., Coleman, M.A., Peterson, L.E., Wyrobek, A., 2003. Gene expression changes in mouse brain after exposure to low-dose ionizing radiation. *Int. J. Radiat. Biol.* 79, 759–775.
- York, J.M., Blevins, N.A., Meling, D.D., Peterlin, M.B., Gridley, D.S., Cengel, K.A., Freund, G.G., 2012. The biobehavioral and neuroimmune impact of low-dose ionizing radiation. *Brain. Behav. Immun.* 26, 218–227. <https://doi.org/10.1016/j.bbi.2011.09.006>
- Yoshida, M., Takayanagi, Y., Inoue, K., Kimura, T., Young, L.J., Onaka, T., Nishimori, K., 2009. Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. *J. Neurosci.* 29, 2259–2271.
- Zambusi, A., Ninkovic, J., 2020. Regeneration of the central nervous system-principles from brain regeneration in adult zebrafish. *World J. Stem Cells* 12, 8.
- Zhang, G., Ferg, M., Lübke, L., Takamiya, M., Beil, T., Gourain, V., Diotel, N., Strähle, U., Rastegar, S., 2020. Bone morphogenetic protein signaling regulates Id1-mediated neural stem cell quiescence in the adult zebrafish brain via a phylogenetically conserved enhancer module. *Stem Cells* 38, 875–889.
- Zhang, Shuhui, Xu, Y., Zhang, Shaozhi, Zhao, C., Feng, D., Feng, X., 2022. Fluorene-9-bisphenol exposure decreases locomotor activity and induces lipid-metabolism disorders by impairing fatty acid oxidation in zebrafish. *Life Sci.* 294, 120379. <https://doi.org/10.1016/j.lfs.2022.120379>
- Zhao, X., Moore, D.L., 2018. Neural stem cells: developmental mechanisms and disease modeling. *Cell Tissue Res.* 371, 1–6.
- Zhao, Y., Fent, K., 2016. Progestins alter photo-transduction cascade and circadian rhythm network in eyes of zebrafish (*Danio rerio*). *Sci. Rep.* 6, 1–10.

- Zimmermann, F.F., Gaspary, K.V., Siebel, A.M., Bonan, C.D., 2016. Oxytocin reversed MK-801-induced social interaction and aggression deficits in zebrafish. *Behav. Brain Res.* 311, 368–374.
- Zimmermann, R., 2006. *La médecine nucléaire: la radioactivité au service du diagnostic et de la thérapie*, EDP Sciences. ed.
- Zupanc, G.K., 2001. Adult neurogenesis and neuronal regeneration in the central nervous system of teleost fish. *Brain. Behav. Evol.* 58, 250–275.

List of FIGURES

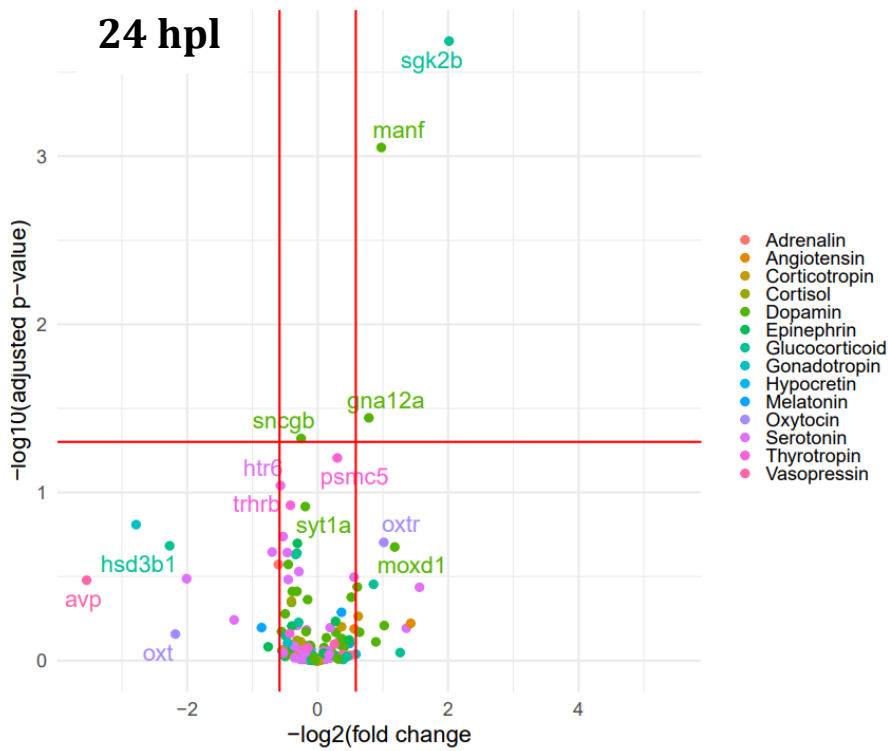
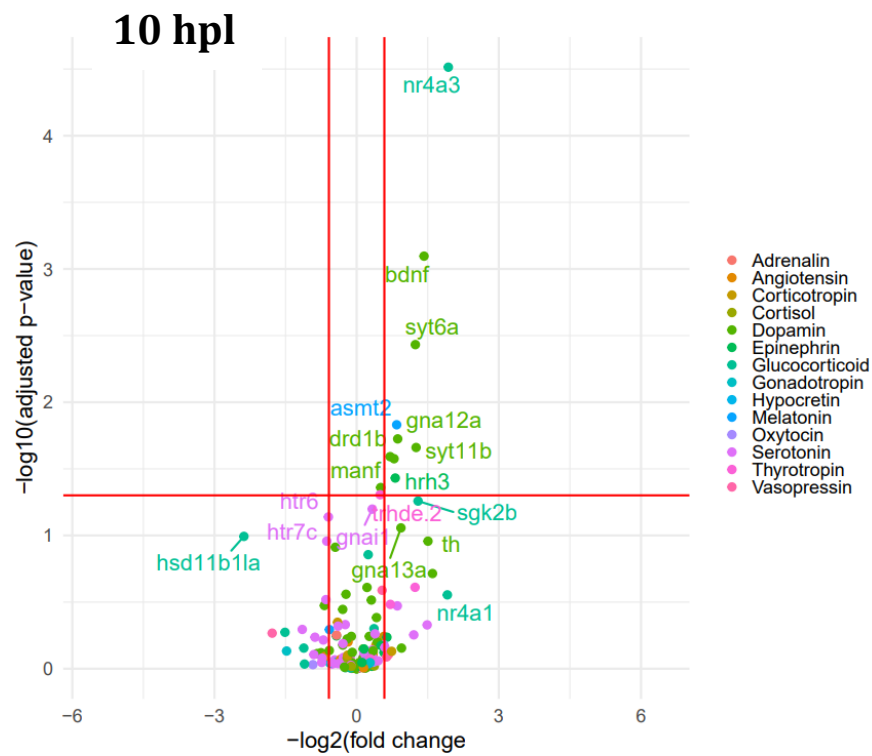
Figure 1 – Cathode tube ray.	21
Figure 2 – First X-ray taken by Wilhelm Röntgen.....	22
Figure 3 – Uranium glass under visible light (left) and under ultraviolet illumination (right).....	23
Figure 4 - Photo of uranium salts that stayed in the drawer taken by Becquerel the 1st of march of 1896.....	24
Figure 5 – The penetration power of ionizing radiation (top figure) and modes of nuclear decay (bottom figure).....	27
Figure 6 – Sampling locations of wild zebrafish specimen.....	30
Figure 7 – (A) The neural tube formation, (B) Development of the telencephalon by inversion (mammals) or eversion (teleost).....	32
Figure 8 – Different models for the subdivision of the forebrain. (A) The columnar model in which the hypothalamus is considered to be the ventral half of the diencephalon, (B) the prosomeric model proposed by Puelles and Rubenstein, (C) a new model proposed by Affaticati et al.	33
Figure 9 - Neural stem cells in development and in the adult.....	35
Figure 10 - Sagittal section of zebrafish brain (A) and neurogenic niches in the brain of mammals (B) and zebrafish (C).	36
Figure 11 – Different cell types at the ventricular zone of the telencephalon. (A) Anterior and (B) medial coronal sections of the telencephalon. (C) Markers of expression of quiescent radial glial cells (Type I), activated radial glial cells (Type II) and neuroblasts (Type III).....	38
Figure 12 - (A) Expression of key genes for the regulation of cell states quiescent NSCs, activated NSCs or neuroblasts in mouse. (B) Signaling pathways to maintain quiescence in NSCs. In green and orange are represented the demonstrated pathways in zebrafish	41
Figure 13: Cellular events occurring after stabwound injury in the telencephalon of zebrafish.....	44
Figure 14 – Social response tested through behavioral experiments.	51
Figure 15 – Connections between habenula of mammals (A) and fish (B) and the social decision-making network.....	53
Figure 16 – Possible oxytocin pathways regarding stress, energy metabolism and social behavior in mammals.	57
Figure 17 - Stress response tested through behavioral experiments	58
Figure 18 – Afferent and efferent neural pathways of the habenula.....	62
Figure 19 – Irradiation Hall	65

Figure 20 – Expression of genes with function in neurogenesis.....	93
Figure 21 – Schematic overview of the molecular and behavioral effects after chronic exposure to low to moderate dose rates of IR.....	95
Figure 22 - Volcano plot of differentially expressed genes after irradiation and stab wound injury (SW) with function in neurotransmission or brain neurohormones.	130
Figure 23 - Schematic overview resuming commonalities and differences between lesion after stabwound and chronic low to moderate dose rates of IR.....	131

List of TABLES

Table 1 – Phylogenetic classification of zebrafish.....	29
Table 2 – Putative homologies between brain regions in the social decision-making network of rodents and teleosts.....	53
Table 3 – Fold change and adjusted p-values (fdr) of genes with function in neurogenesis after irradiation and after induction of brain regeneration by stab wound.	128

ANNEX



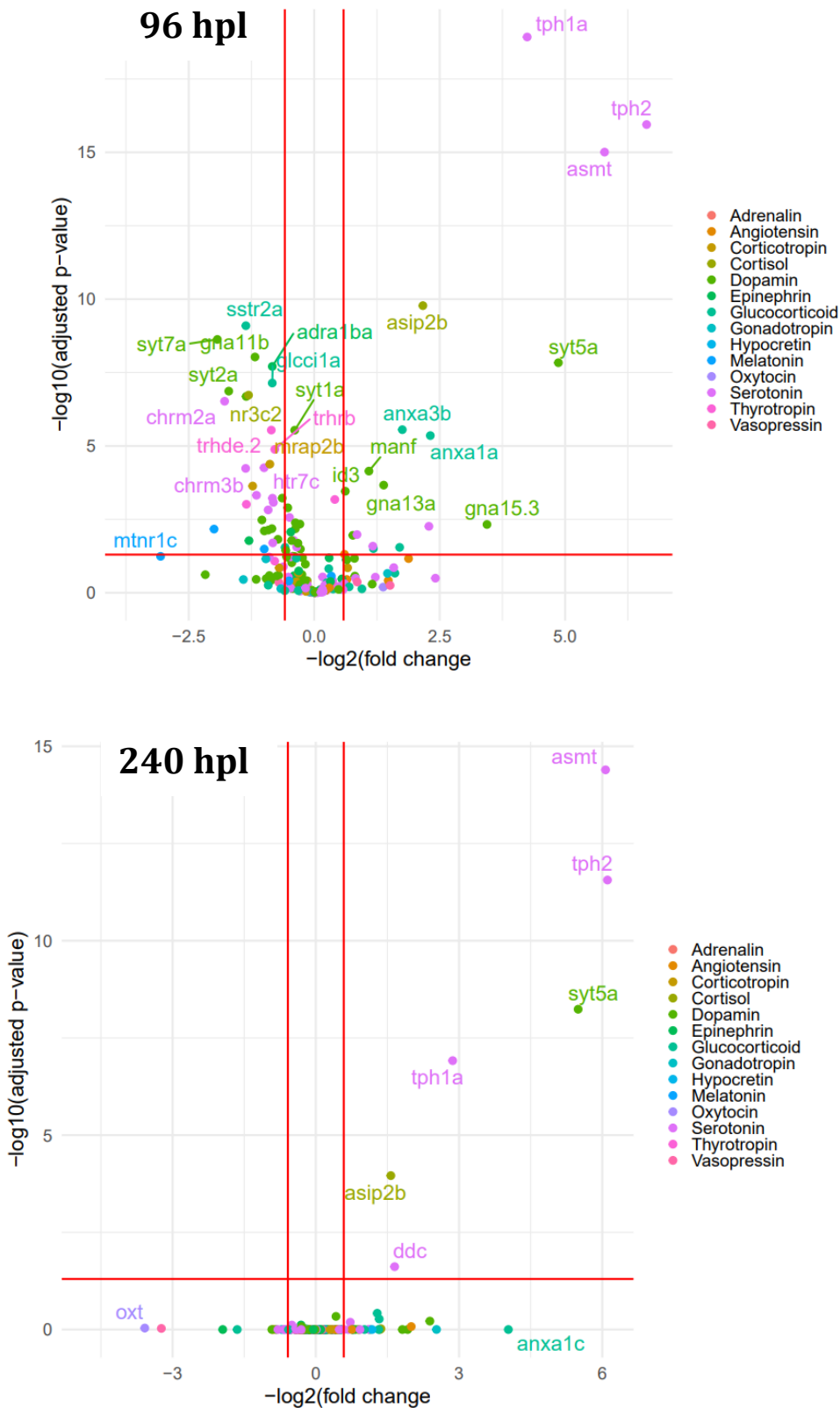


Figure A1: Dysregulated genes with function in neurotransmission or neurohormones at 10 hpl, 24 hpl, 96 hpl and 240 hpl.

