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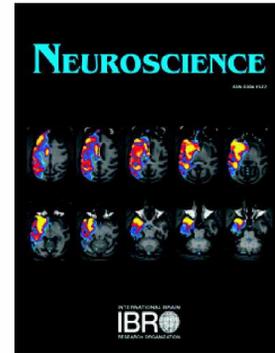


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Repeated potassium iodide exposure during pregnancy impairs progeny's brain development

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ABSTRACT

Protracted radioiodine release, may requires repeated intake of potassium iodide (KI) to protect thyroid gland. It is well established that iodine excess inhibits transiently the thyroid function. As developing foetus depends on maternal thyroid hormones (TH) supply, more knowledge are needed about the plausible effects that repeated KI intake can cause in this sensitive population, especially that even subtle variation of maternal thyroid function may have persistent consequences on progeny brain processing. The aim of this study is to assess

the consequences of repeated intake of KI during pregnancy on the progeny's thyroid function and brain development. **To do so** pregnant *Wistar* rats received KI over eight days, and then thirty days after the weaning, male progeny was subjected to behaviour test. Pituitary and thyroid hormones level, anti-thyroid antibodies level, organs morphology, genes expression and global DNA methylation were assessed. Thirty days after the weaning, KI-exposed male progeny showed an uncommon hormonal status, characterized by a decrease of both TSH (-28%) and FT4 (-7%) levels. Motor coordination was altered in KI-exposed male progeny, at the cerebellar level we observed a decrease of mRNA expression of DCX (-42%) and RC3 (-85%), on the other hand at the cortical level mRNA expression of MBP (+71%), MOBP (+90%) and Kcna1 (+42%) was increased. **To conclude**, repeated KI prophylaxis is not adequate during pregnancy since it led to long-term irreversible neurotoxicity in the male progeny.

KEY WORDS: Behaviour test – potassium iodide – gestation – neurodevelopment – thyroid hormones – progeny.

INTRODUCTION

Electric power production generates large amounts of radioiodine (I-129, I-133, I-131...) as a by-product of uranium fission. In the case of nuclear reactor emergency which involves the release of radioactive material, radioiodine can be accumulated into the thyroid gland by different way as inhalation or ingestion of contaminated products (Braverman et al., 2014). After the Chernobyl disaster, that generates a huge amount of I-131, the incidence of thyroid cancer and thyroid abnormalities spread in the contaminated area (Ron, 2007). These effects are due to β radiation emitted during I-131 decay process. It is well-known that the prenatal phase is critical for the foetus development, the exposure to radiation during this period is responsible for the appearance of thyroid cancer in the progeny (Hatch et al., 2009). The oral

intake of potassium iodide (KI) is recommended by competent authorities as an efficient countermeasure. KI ensure thyroid gland protection essentially through the Wolff-Chaikoff effect, the isotope dilution and the excretion that reduce the committed radioactive dose to the thyroid (Sternthal et al., 1980). World health organization (WHO) recommends a unique administration of KI at 130 mg in adult, pregnant and breastfeeding; fractions of this quantity are considered in other sub-populations. In extreme situations, KI intake can be renewed one more time only for adult (WHO 2017). Both Chernobyl and Fukushima nuclear reactor accidents highlighted the limits of the current iodine thyroid blocking (ITB) policy. It is clear that a unique administration of KI cannot protect in satisfactory way the population. In situations of protracted radioiodine release, repeated KI intake may be necessary for adequate protection of the thyroid gland and its function resumed in the production of the thyroid hormones. These hormones are crucial for foetal neurodevelopment (T. and J., 2004). Their production is under thyroid-stimulating hormone (TSH) control, and requires a complex process; firstly iodide transport through sodium-iodide symporter (NIS) and pendrin (PDS), after that iodide organification which involves thyroid peroxidase (TPO), and H_2O_2 produced by dual oxidase (DUOX), and finally TH transport through monocarboxylate transporter 8 (Carvalho and Dupuy, 2017). In brief, TH production involves several actors, but iodide uptake is the key element of this process. Iodine is found in the environment as a trace-element, exogenous sources (seafood, iodinated salt...) guarantees an adequate supply of iodine for TH synthesis (Rasmussen et al., 2009). According to the WHO, the daily iodine consumption should be around 150 μg in adults (Zimmermann and Andersson, 2012). Otherwise, in specific situations as pregnancy, daily iodine consumption should be around 250 μg (Zimmermann, 2012); this increase is related to the multiple variations that occur during pregnancy as the transfer of maternal thyroid hormones to foetus through placenta, enhanced maternal renal clearance as well as the increased chorionic gonadotropin hormone and T4-binding proteins levels (Serrano-Nascimento et al., 2017b). The involvement of maternal TH in different step of foetal development is well-established (Howdeshell, 2002), especially that foetal thyroid gland is functional only at the second trimester of gestation. The deleterious effects of maternal iodine deficiency (ID) on the foetus development are clearly reported including cretinism and growth retardation (Cao et al., 1994, Niwattisaiwong et al., 2017, Farebrother et al., 2018). Contrariwise, the effect of iodine excess (IE) during pregnancy still poorly explored, It was demonstrated that IE during pregnancy, led to a disturbance of maternal thyroid function and as consequence the occurrence of hypothyroidism and hypothyroxinemia, which has a deleterious impact on the development of

the offspring. (Shi et al., 2015, Pearce et al., 2016). In our previous work, we demonstrated the safety of repeated KI intake in adult rats (Lebsir et al., 2018), Studies regarding the effect of repeated iodine intake during pregnancy are scarce (Noteboom et al., 1997), the aim of this study is to characterize the effects of repeated KI intake during pregnancy on the thyroid function and on the neuromotor development of the progeny using female *Wistar* rats.

MATERIALS AND METHODS

Animal experimentation was approved by the Animal Care Committee of the Institute of radiation protection and nuclear safety (IRSN) and conforms to French regulations (Ministry of Agriculture Act No. 87-848, October 19, 1987, modified May 29, 2001)

Animals and treatment

NaCl (pH 7.4) and KI (1 mg/kg) solutions were prepared and kindly provided by the Central Pharmacy of Armed forces (Orleans, France).

The study included sixteen pregnant *Wistar* rats (Charles River laboratories, L'arbresle, France), divided into control group receiving NaCl solution, and treated group receiving KI 1 mg/kg over eight days since the 9th gestational day (GD9 – GD16). They were housed individually upon arrival and allowed to recover from transportation for one week. Rats were kept in regular light/dark schedule (12h/12h), at $21 \pm 2^\circ\text{C}$ and $50 \pm 10\%$ humidity. Food 0.3 mg I / kg of pellet (A04-10 SAFE, Augy, France) and water were freely accessible. After birth and weaning, male pups were randomly separated from their mother almost one pup from each mother to avoid inbreeding bias. After that, they were divided into control progeny not exposed *in utero* to KI, and treated progeny exposed *in utero* to KI, each group include fourteen animals.

Samples collection

Thirty days after the weaning, progeny were transferred to individual metabolic cages for 24 h, getting access to diet and water *ad libitum*. Urine was collected and centrifuged (3,000 rpm / 10 min); supernatant was frozen at -80°C . Blood was collected by intracardiac puncture

under isoflurane anesthesia (Abbot France, Rungis, France) and then centrifuged (3,000 rpm / 10 min); the plasma supernatant was immediately frozen at -80°C. Thyroid, cortex and cerebellum were instantly deep-frozen in liquid nitrogen and then stored at -80°C.

Biochemical parameters assessment

Plasma and urine biochemical parameters presented in (Appendix Table2), data were measured using an automated spectrophotometric system Konelab 20i (Thermo Fisher Scientific, Cergy-Pontoise, France), with the manufacturer's biological chemistry reagent (Brahms, Asnières sur Seine, France). Phospholipid B (Diagnostic partners, Bormes Les Mimosas, France) indicators were adapted on the spectrophotometric system.

Thyroid markers

Plasma thyroid-stimulating hormone (TSH) was determined with the TSH rat ELISA kit from MP Biomedicals (Illkirch-Graffenstaden, France). Plasma free triiodothyronine (FT3) and free thyroxine (FT4) levels were determined by immunoassay on an IMMULITE[®] 2000 system from Siemens (Saint-Denis, France). Plasma anti-thyroid antibodies were determined with anti-thyroid peroxidase antibody (Anti TPO) bioassay[™] ELISA kit (Rat) and anti-thyroglobulin antibody (ATGA/ TGAB) bioassay[™] ELISA kit (Rat), from US Biological life science (Swampscott, Massachusetts, USA). The analytical sensitivities of TSH, FT4, FT3, Anti-TPO and Anti-Tg are 0.1 ng/mL, 2.83 pmol/L, 1.5 pmol/L, 1 ng/mL and 1 ng/mL respectively.

Real-time PCR

Total RNA was extracted from both thyroid lobes, an average 25 mg of cortex and cerebellum (N = 9 / group), using mirVana[™] miRNA Isolation Kit (Ambion, cat.no.1560). 1 µg of total RNA was reversely transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Courtaboeuf, France) according to the manufacturer's instructions. Real-time qPCR was performed with quantStudio 12K Flex Real-Time PCR System (Thermo

Fisher scientific, Cergy Pontoise, France). Taqman primers (applied biosystems, Courtaboeuf, France) were used to analyse the mRNA levels in thyroid (Lebsir et al., 2018), cortex and cerebellum (Appendix Table 1). Relative changes of genes mRNA expression in KI exposed samples were calculated using $2^{-\Delta\Delta Ct}$ method; GAPDH and ACTB were used as internal controls. Results are expressed as mean \pm SEM.

Global DNA methylation

DNA was extracted from 25 mg of cortex and cerebellum (N = 9 /group) using the QIAmp Mini kit (Qiagen, France) according to the manufacturer's instructions. Methylation profile in the cerebellum and cortex was assessed using 5-mC DNA ELISA kit (Zymoresearch, California, USA). The methylation level was expressed as a percentage. Negative and positive controls were included in each run, according to manufacturer's instruction. The absorbance was measured at 405 – 450 nm using TECAN reader (Infinite[®] 200 PRO NanoQuant Plate Reader).

Histology and immunohistochemistry

Histological and immunohistochemistry analyses were done by an independent laboratory (Biodoxis, Romainville, France). Thyroids, and brains (N = 5 / group) collected at necropsy were placed in a neutral 10% formalin solution to allow immersion fixation. Each individually fixed tissue was then trimmed, placed in a histological cassette, and impregnated with paraffin. For each sample, three 4 μ m thick sections spaced by more than 250 μ m from each other were then stained with hematoxylin and eosin. Detection of Ki67 by immunohistochemistry was performed on thyroids. Paraffin sections were deparaffinized and antigen retrieval was performed in a water bath using the "DAKO Target Retrieval Solution". Immunohistochemical staining was then performed, after blocking endogenous peroxidases (Dako EnVision[®] + System-HRP (DAB), Peroxidase Block), slides were incubated with the primary antibody Rabbit polyclonal anti-Ki67 (Abcam ab15580) for 1 hour at room

temperature. Slides were then rinsed and incubated with secondary antibody (EnVision® +/HRP Rabbit (DAB+) (Dako K4011) for 30 minutes. The signal was then revealed using a DAB solution (Dako EnVision® + System-HRP (DAB), DAB + Substrate Buffer / Liquid DAB + chromogen, 5 min incubation). Finally, counter-staining with Mayer's hematoxylin was performed. Negative controls were performed by substituting the primary antibody by a control isotype IgG rabbit polyclonal (Dako, X0936) or by using diluent alone.

The complete digitization of the tissue sections was performed by the Hamamatsu Nanozoomer. Follicular epithelium nuclei were individually segmented and classified as positive or negative nuclei for the Ki67 cell proliferation marker. This classification was done through thresholding for positive or negative kernels combined with a set of filters based on the morphology and size of segmented objects, thus preserving only objects with a distinctive morphology. A minimum of more than 6000 nuclei were analysed for each thyroid. The percentage of positive nuclei was reported for each animal.

Behaviour tests

Rota-rod test

This apparatus is a test of motor coordination and motor learning, thirty days after the weaning rats were put on the rotating rod of a rota-rod (Ugo Basile, Italy), rotation was set at a steady velocity of 5 rpm for 3 min. Latency to fall off was scored automatically with infrared sensors. This trial was repeated twice again with a lapse-time of one hour between each trial (Marques et al., 2014).

Forced swim test

The apparatus used for this test was a white plastic tub (diameter = 40 cm, height = 60 cm) filled to a height of 48 cm with water at a temperature of 24 – 26 C°. One swim session lasting ten minutes was conducted. The animal's behaviour was assessed each minute but only the immobile time during the last five minutes was recorded and then analysed,

(immobility was taken into account when rat floated in an upright position and made only minimal movements to keep its head above water) (Bourin et al., 2005).

Y maze test

The maze consisted of polyvinyl plastic and had three arms (70 cm in length, 50 cm in height, 10 cm wide at the bottom, and 20 cm wide at the top); the maze was equipped with both intra and extra cues. This test was performed to evaluate the spatial memory of the rats. Rats were placed in the centre of the maze. And then, allowed to move freely for ten minutes. The sequences of arm entry were counted manually to calculate the total number of entries and the alternation percentage (Srivastava et al., 2018).

STATISTICAL ANALYSIS

Descriptive results are expressed as mean \pm SEM. The statistical inference testing was performed using a resampling-based multiple comparison (Westfall and Young, 1993) for a nonparametric p-value adjustment. This approach ensures simultaneously an exact inference procedure despite non-normal characteristics of the data, and unknown covariance structures as well as a multiple testing correction on each multiple response outcome (Bretz et al., 2011). These analyses were conducted using the “multtest” Bioconductor package (Pollard et al., 2005) with the R program (R Core Team and R Foundation for Statistical Computing, 2017). Differences were considered statistically significant when $p \leq 0.05$.

RESULTS

In-utero repeated exposure to KI did not impair the general health parameters and the biochemical status of the progeny

At PND51, body weight of male progeny was not affected by the *in utero* exposure to KI. Thyroid organ coefficient judged by thyroid weight / body weight as well as brain organ coefficient judged by brain weight / body weight were similar between KI exposed and not exposed groups. Plasma markers of liver, kidney and heart functions were equal between

treated and control rats. Urine markers reveal an increase of potassium level in the treated group (Appendix Table 2).

In-utero repeated exposure to KI impaired the activity of the pituitary-thyroid axis of the progeny

As shown in the Table 1, we observed a decrease of TSH and FT4 ($p \leq 0.05$) levels in the KI-exposed group in comparison to control group. FT3 level was similar between both groups. Antithyroid antibodies levels were not affected by the treatment. The application of multiple comparison result in a downward trend of TSH and FT4 (Appendix Table3)

In-utero repeated exposure to KI did not impair the expression of thyroid genes

The expression of genes involved in iodine metabolism and thyroid hormones synthesis did not differ between the male progeny exposed and non-exposed to KI during pregnancy (Figure 1).

In-utero repeated exposure to KI impaired the expression of brain genes

At the cerebellum, we observed a decrease of the mRNA expression of DCX and RC3 ($p \leq 0.05$) in the KI-exposed group compared to control group (Figure 2A). On the other hand, at the cortical level we get an increase of the mRNA expression of MBP, MOBP ($p \leq 0.05$) and Kcna1 ($p \leq 0.01$) in comparison to controls (Figure 2B). The application of multiple comparison result only in an upward trend of Kcna1 (Appendix Table3).

In-utero repeated exposure to KI did not impair the global DNA methylation

The percentage of DNA methylation of cerebellum ($4.07 \pm 0.15\%$ vs $4.01 \pm 0.18\%$) and cortex ($3.79 \pm 0.13\%$ vs $3.81 \pm 0.09\%$) was similar between KI-exposed group and control group (Figure 3).

In-utero repeated exposure to KI did not impair thyroid and brain histology of the progeny

Hematoxylin and eosin staining revealed no alteration attributable to *in utero* exposure to KI in thyroid (figures 4) and brain (figure 5). Ki-67 staining showed that the proliferation rate

was not different for both groups (figure 4), an average of $3.51 \pm 0.60\%$ and $2.70 \pm 0.52\%$ of Ki-67 positive nuclei for treated and untreated rats respectively.

In-utero repeated exposure to KI impaired motor coordination of the progeny

The data showed that the KI-exposed group spent less time on the rota-rod compared to control group. Rats from control group spent more time on the apparatus in the second trial compared to the first trial ($p \leq 0.001$) and in the third trial in comparison to the first ($p \leq 0.001$) and second trial ($p \leq 0.05$). On the other hand, rats from the KI exposed group spent more time on the rota-rod only in the third trial compared to the first trial ($p \leq 0.05$); we did not notice a difference neither between the second and the first trials, nor between the third and the second trials (Figure 6). This result was maintained when we applied multiple comparison (Appendix Table3).

In-utero repeated exposure to KI did not impair spatial memory and depressive-like behaviour of the progeny

KI exposed rats explored the arms of the Y maze in the same way as control rats; the percentage of alteration between different arms was similar between both groups ($73.99 \pm 3.08\%$ vs $69.21 \pm 2.70\%$), (Figure 7A). The immobile time in the forced swim test did not differ between treated rats and controls ($33.36 \pm 3.11s$ vs $31.21 \pm 6.51s$), (Figure 7B).

DISCUSSION

The protection of the foetal thyroid from radioactive iodine has priority in case of nuclear emergency (Verger et al., 2001). Clinical studies have demonstrated that children are at higher risk of inhibition of thyroid function in response to pharmacological dose of stable iodine (Chabrolle and Rossier, 1978, Smerdely et al., 1989), as the degree of prematurity of newborns is related to an increased sensitivity to iodide-induced hypothyroidism; the foetus are expected to be more sensitive to this effect. The safety of repeated administration of potassium iodide during gestation is not very-well documented (Noteboom et al., 1997). In this work, to deal with situation of protracted exposure to radioactive iodine, we developed an

experimental model to evaluate the consequences of repeated KI intake during pregnancy on the male foetal brain development. Thirty days after the weaning, KI-exposed male progeny have a good general health status; their body and thyroid weight were not different from those of non-exposed group. This result comply with other studies that did not find an impact of intrauterine exposure to high iodine levels (I 0.01%, NaI 0.6 mg/L) on foetus body and thyroid weights (Colzani et al., 1999, Serrano-Nascimento et al., 2017a). The global biochemical parameters assessed at plasma and urine levels were comparable between KI-exposed and non-exposed groups. We observed only a slight increase of urinary potassium amount, which does not exceed the biological range, and probably does not have a pathological significance. On the other hand, both TSH and FT4 levels were decreased in the KI-exposed group. This atypical hormonal pattern may indicate a trend to develop a central hypothyroidism in this group owing to hypothalamic or pituitary failure, because TSH level inappropriately decreased instead of increased in response to the low FT4 level (Clemens et al., 2011). The data from literature regarding the effect of excess iodine on the progeny hormonal status are controversial. Serrano-Nascimento et al (2017) found a drop of T3 and T4 levels and a raise of TSH level of the progeny at post-natal day 90 (PND90) in response to excess exposure to iodine during pregnancy and lactation. Whereas, Zhang et al (2012) did not report any modification of thyroid hormones and TSH level of the progeny at PND 45 in response to high iodine exposure (KIO_3 460 $\mu\text{g.L}^{-1}$) during pregnancy and lactation (Zhang et al., 2012). Colzani et al (1999) also did not report an impact of excess iodine exposure (I 0.01%) during pregnancy on the TSH level in term foetus. Since it has been described that excess iodine is linked to thyroid autoimmunity (Luo et al., 2014), we selected two biomarkers of thyroid autoimmunity status anti-TPO and anti-Tg. Our results showed that anti-thyroid antibodies level of KI-exposed progeny did not differ from those of non-exposed ones. To go further, we assessed the impact of *in utero* repeated exposure to KI on the

expression of genes involved in thyroid hormones production; our results did not show a variation of the progeny thyroid genes expression. By contrast, Serrano-Nascimento et al (2017) found that high iodine exposure during pregnancy and lactation reduced the expression of differentiation genes in the thyroid of the progeny. Moreover, in our conditions progeny's thyroid follicles size, shape and cells proliferation index were similar between KI-exposed and non-exposed groups. These results are in agreement with the genes expression result as we did not report any variation of thyroid differentiation genes especially thyroglobulin. On the other hand, Serrano-Nascimento et al (2017) reported a reduction of the thyroid follicle's diameter and decreased colloid content within the follicles of iodine-exposed progeny.

Thyroid auto-immunity status, genes expression and morphology results reinforced our hypothesis and suggest that the reduction of thyroid hormone FT4 level is not related to an impact of iodine exposure during pregnancy on the foetal thyroid. We suspect an impact on the progeny's brain. We selected PND51 as crucial time point in the brain development of the rat (Babikian et al., 2010) to explore the effect of excess iodine exposure during pregnancy on the expression of genes in two key structures of the brain, the cerebellum and the cortex. The rat brain at PND51 is considered developmentally equivalent to the late teenager stage. Our results at cerebellar level demonstrated a decrease of the mRNA expression of DCX a microtubule-associated protein essential for normal neural development, widely expressed in migrating neurons during embryonic and postnatal development (Francis et al., 1999, Gleeson et al., 1999). Dcx is considered as an important marker of new neurons, which mediates neuronal migration by regulating the organization and stability of microtubules (Horesh et al., 1999, Friocourt et al., 2003). Reduced DCX expression during development is related to the appearance of brain pathologies (Piens et al., 2010). It was demonstrated that iodine deficiency and hypothyroidism reduced DCX expression in rats hippocampus from PND14 onward (Gong et al., 2010). On the other hand, it was demonstrated that iodine deficiency and

hypothyroidism had a biphasic effect on the expression of DCX in the cerebellum regarding the developmental window, with a reduction of DCX expression on PND 14, and an increase of its expression on PND 21 and PND 28 (Wang et al., 2012). We also reported a decrease of the mRNA expression of RC3, a brain-specific gene involved in synaptic plasticity as well as learning and memory (Singec et al., 2003). The RC3 expression clearly depends on thyroid hormones and is regulated *via* the genomic molecular mechanism of thyroid hormone in the rat brain (Royland et al., 2008). RC3 is expressed in the cerebellar purkinje cells (Larouche et al., 2006). Other studies found a relationship between the maternal or foetal hypothyroidism and a decrease of the expression of RC3 in the cortex and hippocampus of rat progeny (Dowling and Zoeller, 2000, Dong et al., 2010, Sawano et al., 2013). At the cortical level, we surprisingly found an increase of mRNA expression of MBP, MOBP and Kcna1. MBP and MOBP are the foremost constituent elements in CNS myelin, and they supposedly function in myelin sheath compaction (Montague et al., 2006), and Kcna1 plays a role in setting resting membrane potential; Neurons in Kcna1 knockout mice are more excitable and have a lower threshold for potassium currents (Brew et al., 2003). It was suggested that the down-regulation of Kcna1 might impede the process of activity-dependent neural wiring during development, subsequently impairing the capacity for learning and memory after maturation (Kobayashi et al., 2009). Contrary to our findings, rat perinatal exposure to propylthiouracil (PTU), a potent thyroid hormone inhibitor, induced a significant decrease in the expression of MBP, MOBP and Kcna1 in the progeny cortex (Kobayashi et al., 2009). The present study demonstrate for the first time that the intrauterine repeated exposure to potassium iodide can regulate the expression of well-characterized thyroid hormone responsive genes in the progeny brain. It has been described that maternal environment during intrauterine period has a critical role in the programming of gene expression mainly through epigenetic pathway. Our data demonstrated that the global DNA methylation at the cerebellar and the cortical levels

was not affected by the intrauterine repeated exposure to KI, also the expression of genes responsible of the process of methylation (Dnmt1 and Dnmt3) and demethylation (TET1 – TET2 – TET3 and TGD) was not affected by the exposure to KI (data not shown). We hypothesised that either the variation of genes expression is not due to epigenetic effect or it is due to another epigenetic mechanism than the global DNA methylation.

The haematoxylin eosin staining did not show a difference between the histology of the brain of KI-exposed and no-exposed progeny. On the other hand, the intrauterine exposure to KI led to an impairment of motor coordination evaluated by the rota-rod test, indicating the functional defect of the cerebellum. This data is consistent with the decrease of cerebellum genes expression, and in accordance with other studies that related congenital hypothyroidism to motor incoordination and to alteration of cerebellum development (Hasebe et al., 2008, Shimokawa et al., 2014). In our conditions, spatial memory and depressive like-behavior, evaluated through Y maze and forced swim test respectively were not affected by the KI exposure. By contrast, Zhang et al. 2012 found that maternal excessive iodine intake impaired spatial memory of pups.

Based on literature data (Zhang et al., 2012, Serrano-Nascimento et al., 2017b) we hypothesised that the maternal repeated exposure to KI led to maternal hypothyroidism or hypothyroxinaemia, which probably led to a pups congenital hypothyroidism and impact the expression of brain TH responsive genes (figure 8).

To conclude, in our experimental conditions repeated administration of KI during pregnancy is not safe for rat male progeny; and induced an irreversible neurotoxicity. Maybe the duration of the treatment was too long or the chosen pregnancy window was not adequate. For that, in the future we will investigate the effect of repeated KI intake during pregnancy at different developmental windows, maternal data will be assessed to verify our hypothesis. Effect on female progeny will also be assessed in the future. Finally, the result of this study

will contribute to the evolution of the current KI marketing authorisation, and to the update of the current iodine thyroid blocking guidelines in the case of protracted radioiodine exposure.

DECLARATION OF INTERESTS

The authors do not report conflict of interests regarding the publication of this paper.

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APPENDICES

Table 1: Taqman primers used for genes expression analysis through Real-Time PCR.

Genes	Assay ID number
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A. Cerebellum	
TNC : Tenascin C	Rn01454948_m1
RELN : Reelin	Rn00589609_m1
DCX : Doublecortin	Rn00670390_m1
RC3 : Neurogranin	Rn01531573_g1
Calm2 : Calmodulin 2	Rn04418658_g1
Camk2d : Calcium/calmodulin-dependent protein kinase II delta	Rn00560913_m1
BDNF : Brain-derived neurotrophic factor	Rn02531967_s1
Hr : hairless	Rn00577605_m1
B. Cortex	
Tbr2 : T-box brain protein 2	Rn01746545_m1
ARPC5 : Actin related protein 2/3 complex, subunit 5	Rn01759260_m1
Arc : Activity-regulated cytoskeleton-associated protein	Rn00571208_g1
MBP : Myelin basic protein	Rn01399619_m1
MOBP : myelin-associated oligodendrocyte basic protein	Rn01445606_g1
MAG : Myelin-associated glycoprotein	Rn01457782_m1
PLP1 : Proteolipid protein 1	Rn01410490_m1
Kcna1 : shaker related potassium channel 1	Rn00597355_s1
CamkIV : Calcium/calmodulin-dependent protein kinase IV	Rn00664802_m1
C. Housekeeping genes	
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase	Rn01775763_g1
ACTB: Actin, beta	Rn00667869_m1

Table 2: Progeny's general and biochemical parameters 30 days post-weaning

Function	Control	KI 1mg/kg
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General indicators	Final body weight (g)	273.90 ± 14.00	276.80 ± 33.00
	TW/BW Ratio	0.065 ± 0.006	0.063 ± 0.009
	BW/BW Ratio	0.007 ± 0.001	0.007 ± 0.001
Plasma biomarkers			
1. Miscellaneous	Cholesterol (mmol/L)	1.77 ± 0.06	1.80 ± 0.05
	Phospholipids B (g/L)	1.53 ± 0.05	1.54 ± 0.05
	Triglycerides (mmol/L)	1.58 ± 0.20	1.52 ± 0.24
	Total proteins (g/L)	65.66 ± 3.43	67.39 ± 3.17
2. Electrolytes	Calcium (mmol/L)	2.95 ± 0.07	3.17 ± 0.08
	Phosphorus (mmol/L)	2.86 ± 0.07	3.02 ± 0.10
	Iron (µmol/L)	47.13 ± 3.00	46.21 ± 2.38
	Chloride (mmol/L)	106.20 ± 1.73	107.19 ± 1.60
	Potassium (mmol/L)	5.10 ± 0.17	5.05 ± 0.15
	Sodium (mmol/L)	138.14 ± 2.40	140.05 ± 2.17
3. Thyroid markers	Anti-TPO (ng/mL)	35.86 ± 5.00	
	Anti-Tg (ng/mL)	556.88 ± 87.04	41.43 ± 4.07 377.78 ± 42.32
4. Liver markers	ALAT (U/L)	29.63 ± 2.31	30.44 ± 3.40
	ASAT (U/L)	91.66 ± 9.64	91.17 ± 3.15
	ASAT/ALAT Ratio	3.29 ± 0.41	3.36 ± 0.33
5. Kidney markers	Creatinine (µM)	46.01 ± 1.81	45.33 ± 0.87
	Urea (mM)	4.47 ± 0.14	4.86 ± 0.23
6. Heart markers	CK (U/L)	517.24 ± 255.53	30.1.03 ± 60.63
	CK-MB (U/L)	375.04 ± 35.05	437.54 ± 16.93
Urine biomarkers			
1. Electrolytes	Chloride (mmol/24h)	5.11 ± 0.85	5.40 ± 0.88
	Potassium (mmol/24h)	0.96 ± 0.08	1.36 ± 0.15*
	Sodium (mmol/24h)	0.96 ± 0.07	1.12 ± 0.08
	Phosphorus (mmol/24h)	0.06 ± 0.03	0.10 ± 0.03
	Calcium (µmol/24h)	66.73 ± 12.40	48.77 ± 5.63
2. Miscellaneous	Urinary proteins (mg/24h)	15.15 ± 1.87	11.06 ± 1.80
	Uric acid (µM/24h)	11.68 ± 0.82	13.67 ± 1.08
	Urea (Mm/24h)	6.57 ± 0.48	8.24 ± 0.77
	Creatinine (µM/24h)	73.63 ± 5.84	81.90 ± 8.60

ALAT: alanine aminotransferase, ASAT: aspartate aminotransferase, TW: thyroid weight, BW: body weight, TSH: Thyroid-stimulating hormone, FT4: free thyroxine, FT3: free triiodothyronine, Anti-TPO: anti-thyropoxydase antibody, Anti-Tg: antithyroglobulin antibody, CK: creatine kinase, CK-MB: creatine kinase myocardial band. TW/BW: thyroid organ

coefficient, BW/BW: brain organ coefficient. Data are expressed as mean \pm SEM (n = 13 /group), * p < 0.05 vs control before multiple comparison.

Table 3: Pvalue before and after multiple comparison

Plasma			Thyroid			Cerebellum		
Parameter	Pvalue	Pvalue adjusted	Parameter	Pvalue	Pvalue adjusted	Parameter	Pvalue	Pvalue adjusted
CK-MB	0.0336	0.4124	NIS	0.369	0.966	RC3	0.0257	0.1505
Urea	0.111	0.7588	AIT	0.862	1	DCX	0.03	0.1687
Calcium	0.1154	0.8036	PDS	0.488	0.986	TNC	0.4012	0.9705
Phosphorus	0.1766	0.9026	TPO	0.657	0.999	RELN	0.4746	0.9889
Proteins	0.212	0.9704	Tg	0.820	1	Hr	0.7152	0.9997
Glucose	0.4066	0.9996	DUOX2	0.717	1	Calm2	0.9813	1
Sodium	0.4542	0.9998	DUOXa2	0.357	0.962	CamK2D	0.9734	1
ALAT	0.9098	1	MCT8	0.754	0.999	BDNF	0.9948	1
ASAT	0.9646	1	Dio2	0.877	1	Behavior		
Cholesterol	0.5564	1	Cortex			Immobility	0.7806	0.9994
CK	0.804	1	Kcna1	0.0102	0.0662	Alternation	0.2304	0.7584
Creatinin	0.9348	1	MOBP	0.0322	0.218	Control trial 1 vs 2	0.0022	0.0072
Iron	0.665	1	MBP	0.0324	0.2235	Control trial 1 vs 3	0	0
Chlorine	0.5996	1	CamkIV	0.0519	0.3957	KI trial 1 vs 2	0.2194	0.5956
Potassium	0.7574	1	MAG	0.0859	0.539	KI trial 1 vs 3	0.0177	0.0620
Phospholipids	0.7888	1	PLP1	0.0888	0.5657			
Triglycerids	0.7516	1	APRC5	0.1722	0.8167			
			RC3	0.2162	0.8745			
			ARC	0.4374	0.9962			
			Tbr2	0.7844	0.9995			
			RELN	0.572	0.9998			
			HOMER1	0.8006	1			

FIGURE CAPTIONS

Figure 1: mRNA expression level of thyroid genes involved in iodine transport (NIS – AIT and PDS), iodine metabolism (DUOX2 –DUOXA2 – TPO and Tg) and thyroid hormone metabolism (MCT8 and Dio2). The results are relative to GADPH and ACTB mRNA level. Data distributions are represented through boxplots (quartiles and minimum/maximum).

Figure 2: (A): mRNA expression level of genes involved in granular cell migration (TNC and RELN), setting up the cerebellum (DCX – RC3 – Calm2 – Camk2d and BDNF), and transcriptional regulation (Hr) at the cerebellum. (B): mRNA expression level of cortical genes involved in transcriptional regulation (Tbr2), myelination (MBP – MOBP – MAG and PLP1), synaptogenesis (ARC – Kcna1 – RC3 – HOMER1 and APRC5), maturation (CamkIV), and neuronal cell migration (RELN) at the cortex. The results are relative to GADPH and ACTB mRNA level. Data distributions are represented through boxplots (quartiles and minimum/maximum). * $p \leq 0.05$, ** $p \leq 0.01$ vs control before multiple comparison.

Figure 3: percentage of global DNA methylation assessed in the cerebellum and cortex of the progeny. The Data distributions are represented through boxplots (quartiles and minimum /maximum).

Figure 4: (A-B): Haematoxylin and eosin histological sections of the thyroid illustrating follicles, (C-D): Immunohistochemical expression of Ki67 positive nuclei in control and KI-exposed rats, respectively (scale bar 250 μ m).

Figure 5: (A-B): Haematoxylin and eosin histological sections of the brain structures: cerebellum, hippocampus and cortex in control and KI-exposed rats, respectively (scale bar 10 mm).

Figure 6: Rotarod experiment including three trials for the assessment of motor coordination and learning. Data distributions are represented through boxplots (quartiles and minimum/maximum). * $p \leq 0.05$ vs trial 1, *** $p \leq 0.001$ vs trial 1 and \$ $p \leq 0.05$ vs trial 2.

Figure 7: (A) Y maze test to assess the spatial memory and (B) forced swim test to assess the anxiety and depression status. Data distributions are represented through boxplots (quartiles and minimum/maximum).

Figure 8: hypothetic mechanism of repeated *in utero* exposure to potassium iodide effects on the male progeny.

HIGHLIGHTS:

- This study evaluates the impact of *in utero* KI intake on the progeny brain processing.
- *In utero* exposure to KI impairs progeny motor coordination
- Our results will contribute to the evolution of iodine guidelines in case of pregnancy.

ACCEPTED MANUSCRIPT

Group	TSH	FT4	FT3
Control	4.80 ± 0.50	21.98 ± 0.38	6.03 ± 0.31
KI	3.47 ± 0.30*	20.48 ± 0.44*	6.84 ± 0.28
P-value	0.030	0.0201	0.120
P-value adjusted	0.088	0.0585	0.309

Table1: HPT axis parameters thirty days post-weaning. Data are expressed as mean ± SEM.

ACCEPTED MANUSCRIPT

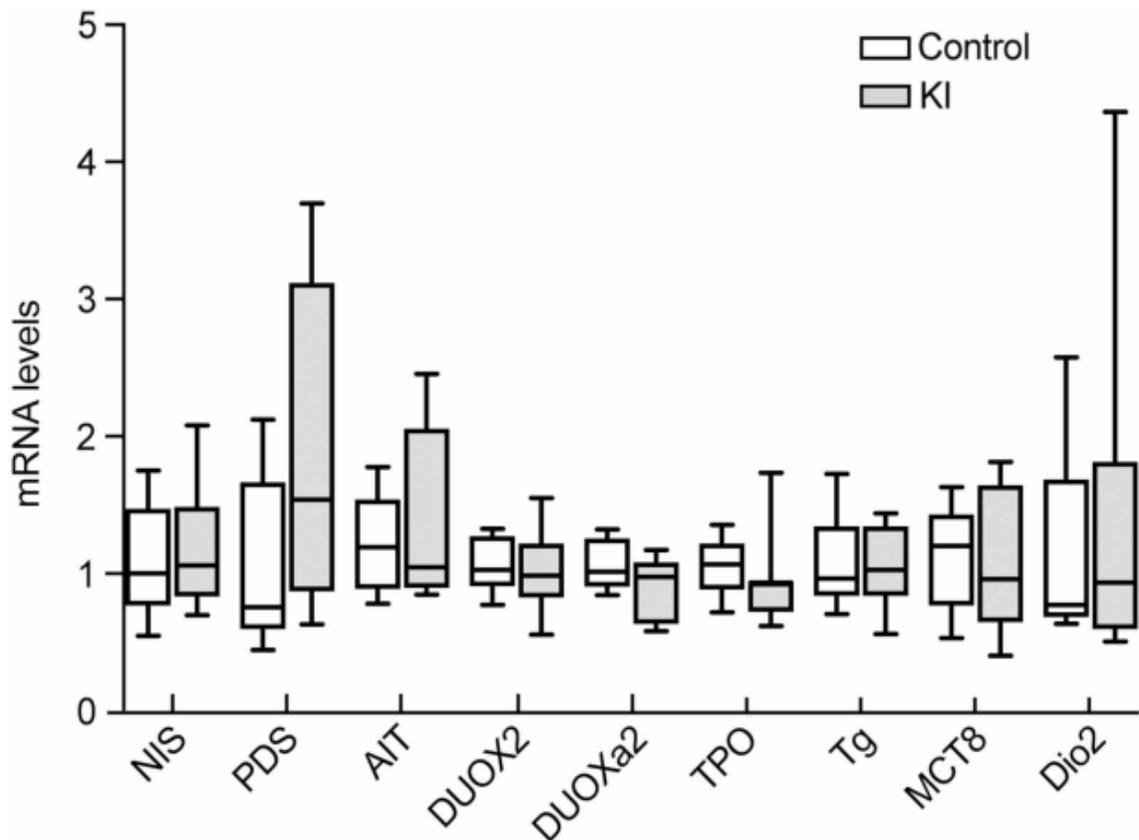


Figure 1

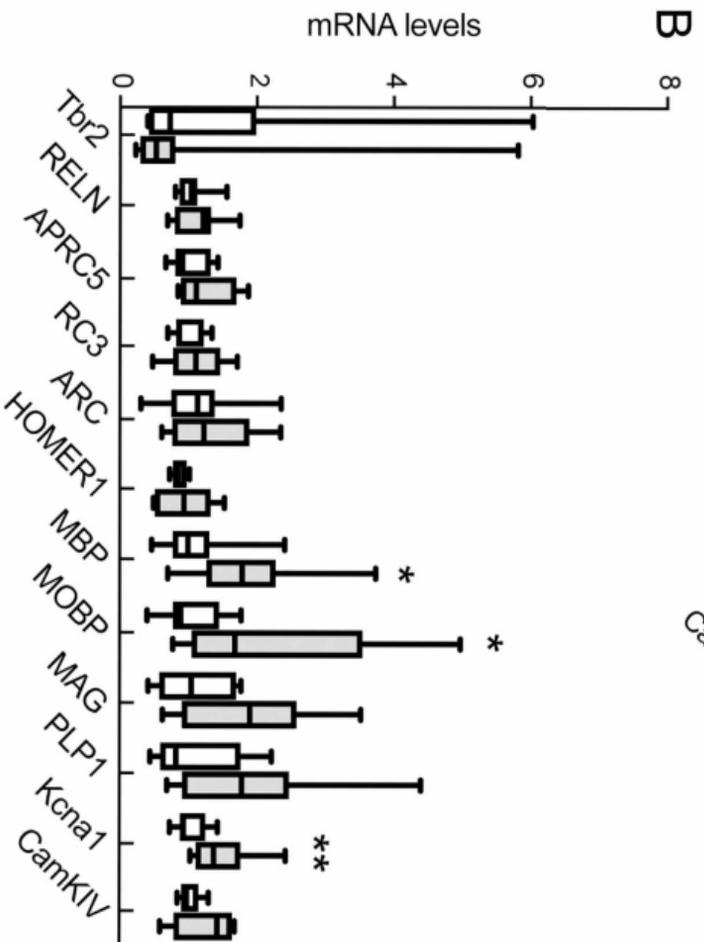
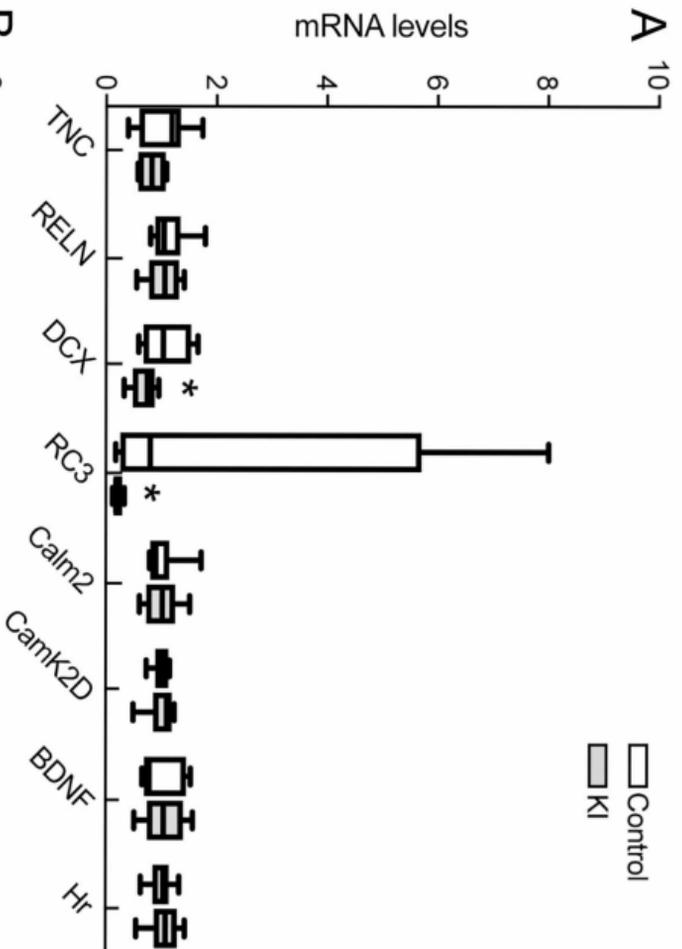


Figure 2

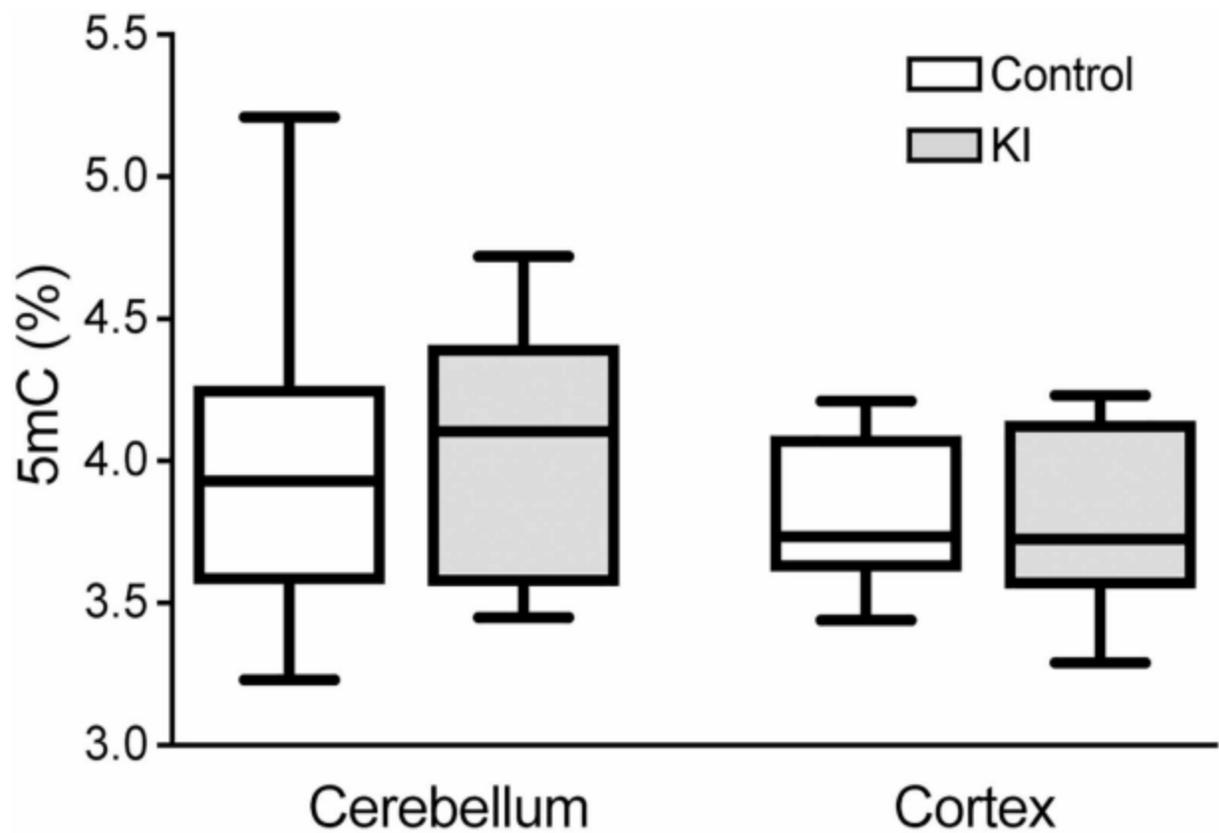


Figure 3

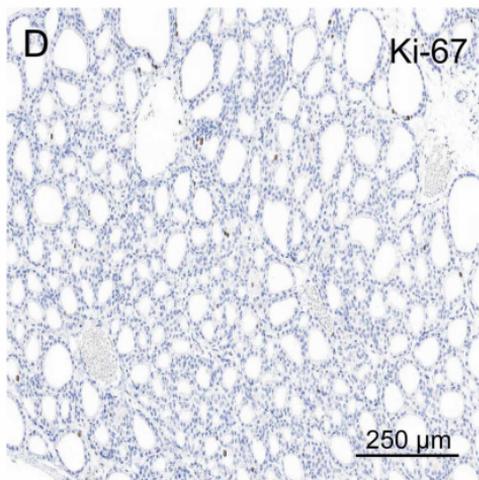
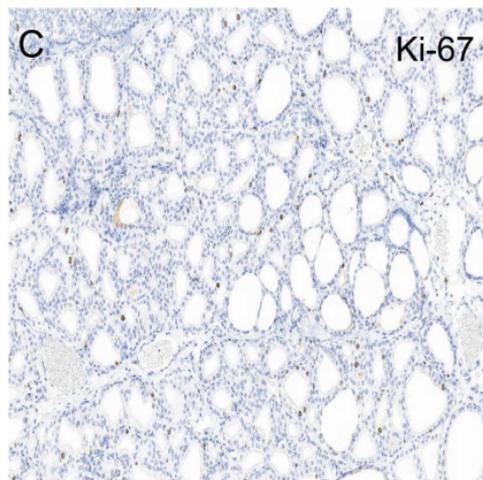
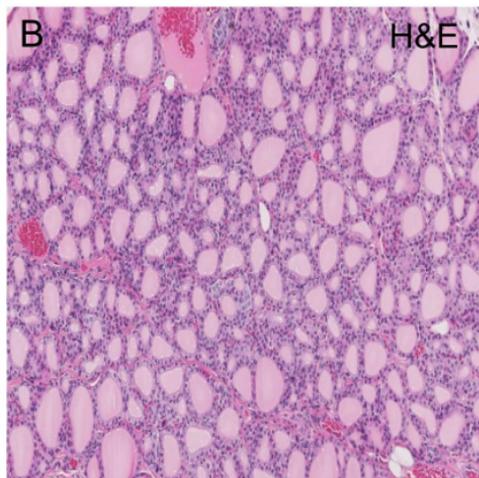
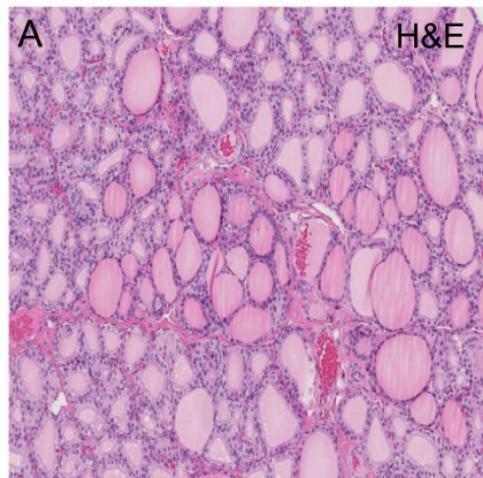


Figure 4

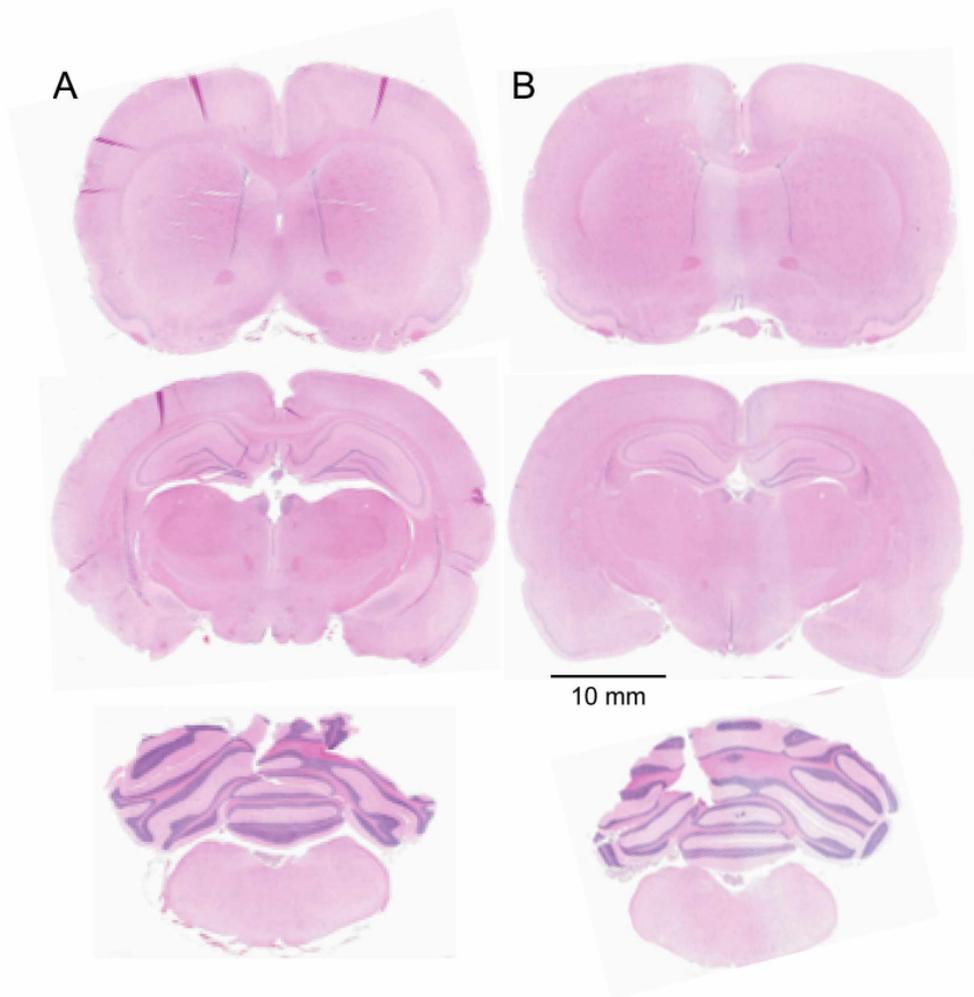


Figure 5

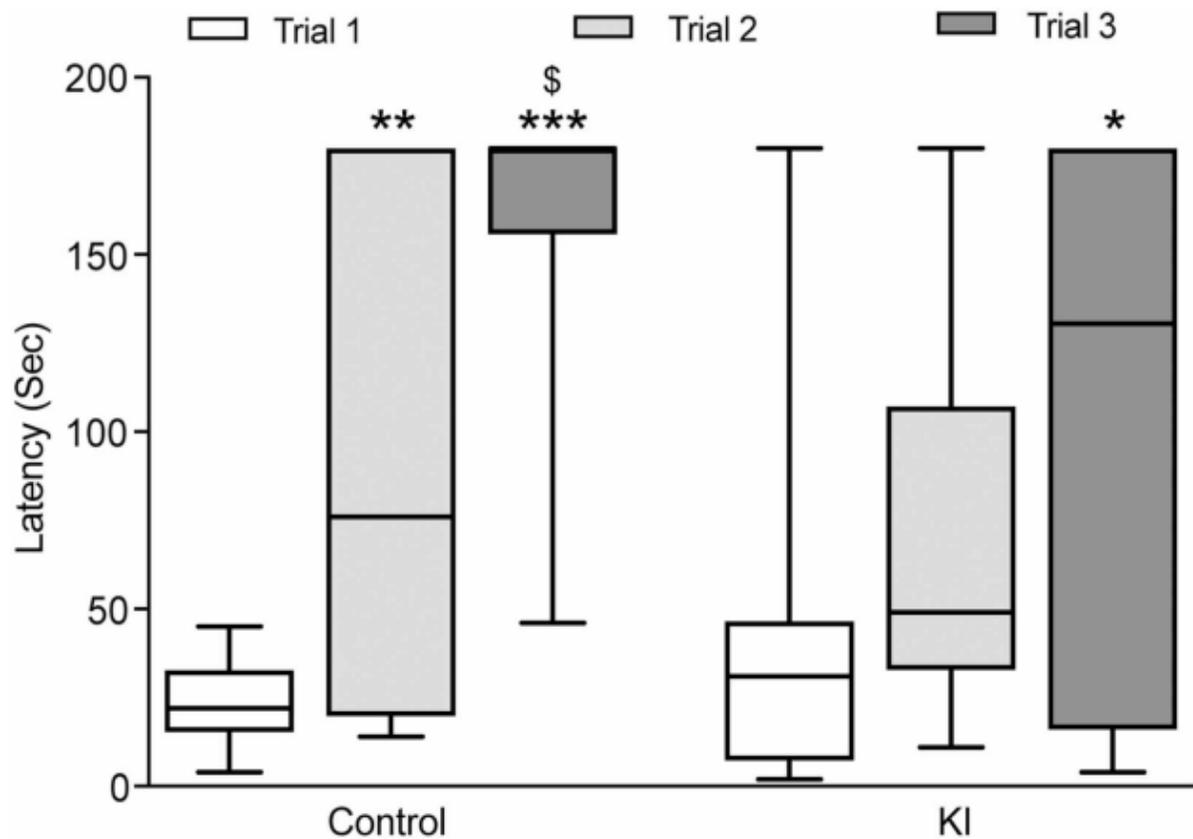


Figure 6

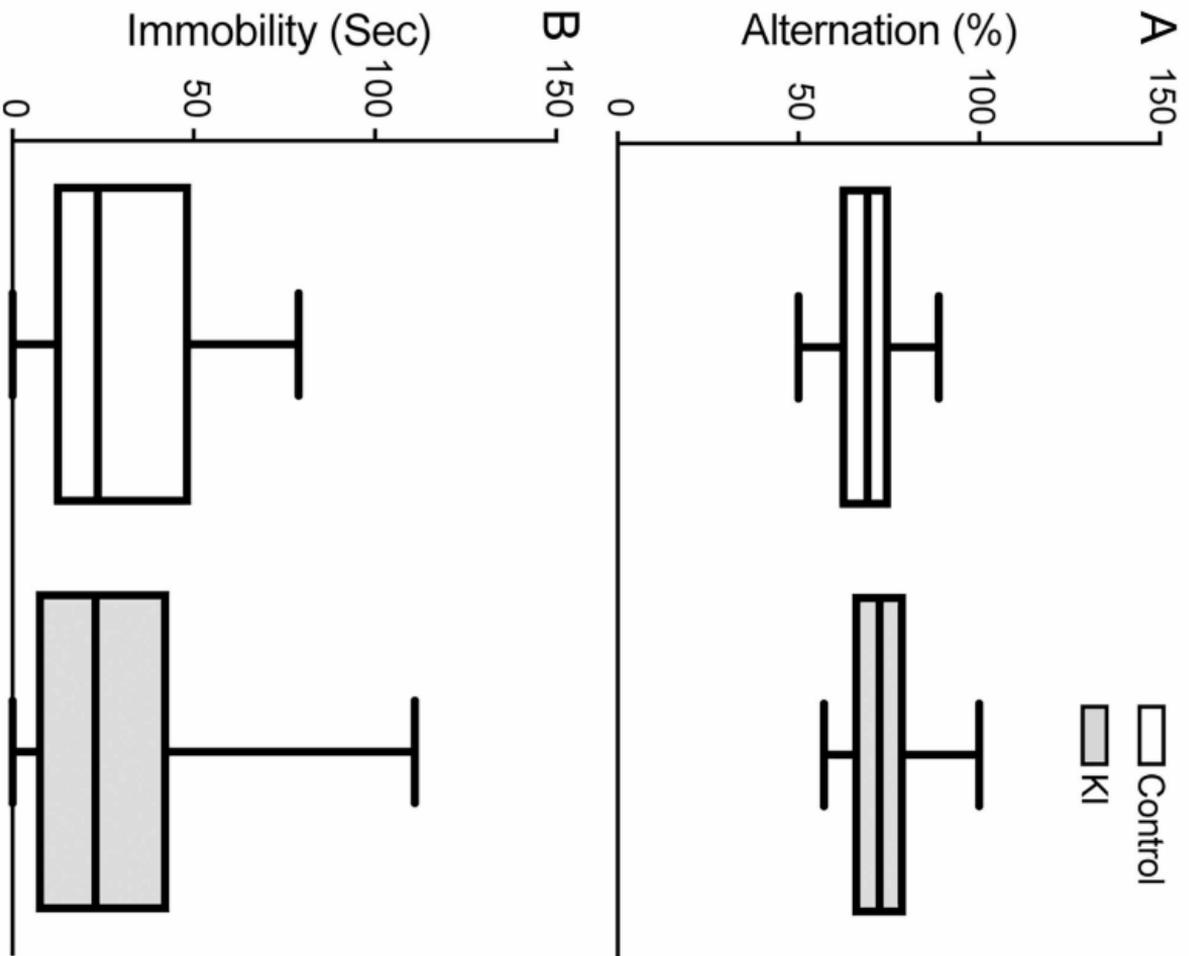


Figure 7

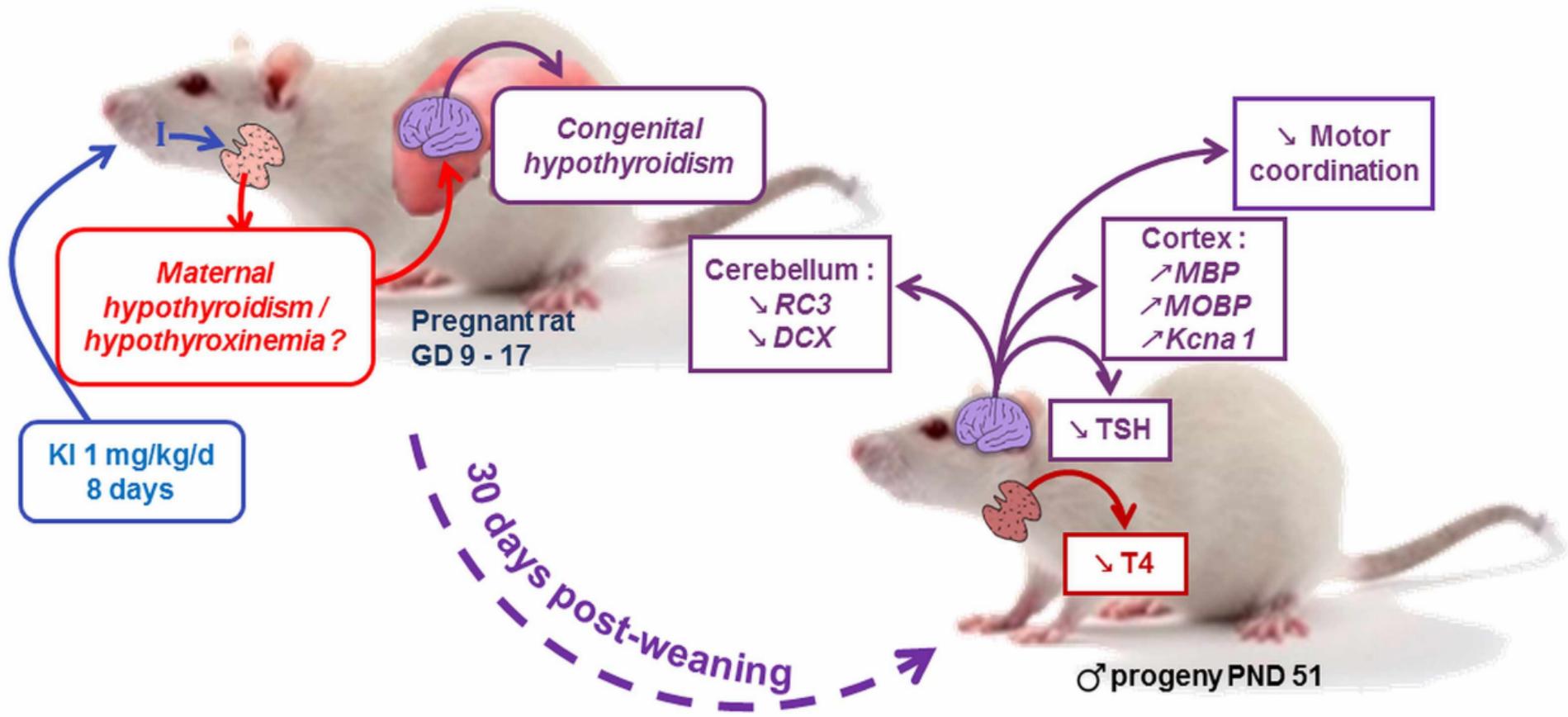


Figure 8