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Bisphenol A induces sex-dependent alterations in the neuroendocrine response of Djungarian hamsters to photoperiod

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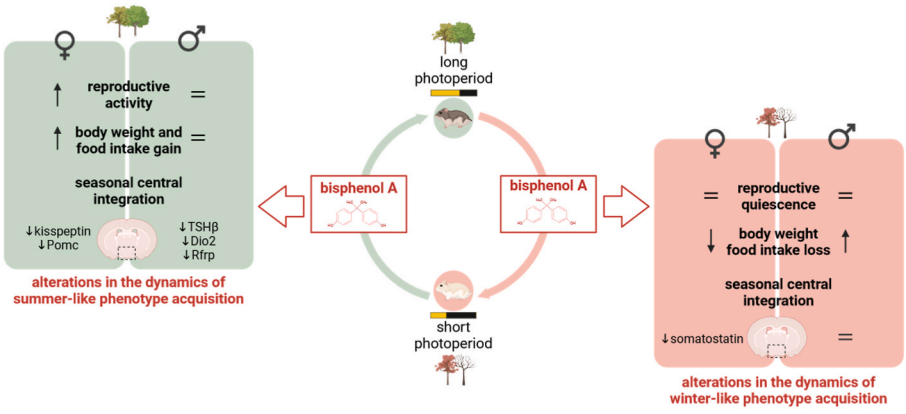
GRAPHICAL ABSTRACT



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ABSTRACT

In nature, species synchronize reproduction and energy metabolism with seasons to optimize survival and growth. This study investigates the effect of oral exposure to bisphenol A (BPA) on phenotypic and neuroendocrine seasonal adaptations in the Djungarian hamster, which in contrast to conventional laboratory rodents, is a well-recognized seasonal model. Adult female and male hamsters were orally exposed to BPA (5, 50, or 500 μg/kg/d) or vehicle during a 10-week transition from a long (LP) to short (SP) photoperiod (winter transition) or vice versa (summer transition). Changes in body weight, food intake, and pelage color were monitored weekly and, at the end of the exposure, expression of hypophysio-hypothalamic markers of photoperiodic (*TSHβ*, *deiodinases*), reproductive (*Rfrp*, kisspeptin) and metabolic (*somatostatin*, *Pomc*) integration, reproductive organ activity, and glycemia were assessed. Our results revealed sex-specific effects of BPA on acquiring SP and LP phenotypes. During LP to SP transition, females exposed to 500 μg/kg/d BPA exhibited delayed body weight loss and reduced feed efficiency associated with a lower expression of *somatostatin*, while males exposed to 5 μg/kg/d BPA showed an accelerated acquisition of SP-induced metabolic parameters. During SP to LP transition, females exposed to 5 μg/kg/d BPA displayed a faster LP adaptation in reproductive and metabolic parameters,

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along with kisspeptin downregulation occurring 5 weeks earlier and *Pomc* upregulation delayed for up to 10 weeks. In males, BPA exposure led to decreased expression of central photoperiodic integrators, with no effect on the acquisition of the LP phenotype. This pioneering study investigating EDCs' effects on mammalian seasonal physiology shows that BPA alters the dynamics of metabolic adaptation to both SP and LP transitions with marked sex dimorphism, causing temporal discordance in seasonal adaptation between males and females. These findings emphasize the importance of investigating EDCs' effects on non-conventional animal models, providing insights into wildlife physiology.

1. Introduction

Among the plethora of adaptations to environmental constraints that species are capable of, physiological adaptations to seasonal changes are particularly remarkable. Indeed, most species living in moderate to high-latitude regions of the world synchronize their metabolic and reproductive physiology to seasonal changes in geophysical factors such as temperature, humidity, and photoperiod. This adaptive process optimizes their energy resource allocation and times the offspring birth at the best period of the year, ultimately guaranteeing the survival of the species (Norris and Jones, 1987; Bronson, 1985; Shinomiya et al., 2014). A typical example of such adaptive processes is observed in the Djungarian hamster (*Phodopus sungorus*), a seasonal rodent that exhibits high food consumption, higher body weight, active reproduction, and a grey-colored fur in spring and summer, while, as winter is coming, it displays reduced metabolic activity, inhibited reproduction, and a white fur (Wade and Bartness, 1984; Warner et al., 2010; Figala et al., 1973; Duncan and Goldman, 1984). The Djungarian hamster is an extensively studied animal model for investigating seasonal physiology, as its natural ability to adapt to seasonal changes can be replicated by modifying day length (photoperiod) in controlled laboratory settings (Goldman, 1999). When reared under long, summer-like, photoperiods (LP), pubertal development starts around 20 days of age in male and 35 days in females, both reaching adulthood by 60–80 days (Adam et al., 2000; Yellon and Goldman, 1984). In laboratory conditions, Djungarian hamsters live for up to three years. In adulthood, a direct transfer to short photoperiod (SP) or a return to LP leads to the development of a winter-like or summer-like phenotype, respectively, within 10–12 weeks (Wade and Bartness, 1984; Hoffmann, 1973; Bernard et al., 1997).

In addition to photoperiod, other seasonal ecological factors such as ambient temperature, humidity, and food availability can influence physiological responses to changing seasons (Eskes, 1983; Kriegsfeld et al., 2000; Larkin et al., 2002; Nelson et al., 1983). However, for mammals living above 10°–20° latitude, variation in daylight duration throughout the year serves as the most reliable seasonal indicator. This is because it is directly tied to the earth's rotation around the sun, making it stable and predictable from year to year (Bronson, 1985; Nakane and Yoshimura, 2019).

Decades of studies using seasonal species such as Djungarian and Syrian hamsters, and sheep unveiled the neuroendocrine mechanisms underlying these photoperiodic physiological adaptations (Dardente and Simonneaux, 2022; Hazlerigg and Simonneaux, 2015). In mammals, annual photoperiodic changes are processed into the brain by a retino-hypothalamic-pineal axis. This results in prolonged synthesis of the nocturnal hormone melatonin during long winter nights (or SP). In contrast, there is a shorter melatonin synthesis during the brief summer nights (or LP) (Hazlerigg and Simonneaux, 2015). This melatoninergic seasonal message is integrated into the *pars tuberalis*, an endocrine structure of the pituitary gland that contains a high density of melatoninergic receptors (Klosen et al., 2002; Dardente et al., 2003). There, thyrotropic cells express the thyroid hormone stimulating beta subunit (TSHβ) which is strongly inhibited by the long melatonin peak in SP (Bockmann et al., 1996; Dardente et al., 2003). In LP, the highly produced TSH activates TSH receptors localized on tanycytes, glial cells of the intracerebroventricular-hypothalamic interface, which in turn regulate the expression of deiodinases, enzymes involved in thyroid hormone metabolism (Tu et al., 1997; Guadaño-Ferraz et al., 1997; Yoshimura et al., 2003). Thus, the LP-induced expression of TSH results in both a higher expression of deiodinases 2 (Dio2) that convert the prohormone thyroxine (T4) to the bioactive thyroid hormone triiodothyronine (T3), and a reduced expression of Dio3 that inactivates T3, whereas the SP-induced inhibition of TSH is associated with an opposite low expression of Dio2 and high expression of Dio3 (Watanabe et al., 2004; Revel et al., 2006; Hanon et al., 2010; Sàenz de Miera et al., 2013; Yoshimura et al., 2003; Helfer et al., 2013). This melatonin-driven TSH control of tanycytic Dios leads to a photoperiodic switch in intra-hypothalamic concentrations of T3, with higher values in LP as compared to SP (Klosen et al., 2013). Through mechanisms yet to be determined, seasonal variations in hypothalamic T3 regulate the expression of the GnRH regulators kisspeptin in the arcuate nucleus (ARC) and RFRP-3 in the dorsomedial hypothalamus (Klosen et al., 2013; Henson et al., 2013; Dardente and Simonneaux, 2022), as well as the ARC growth hormone regulator somatostatin (Herwig et al., 2012, 2013; Petri et al., 2014; Klosen et al., 2013) and appetite regulator pro-opiomelanocortin (POMC) (Reddy et al., 1999; Mercer et al., 2000, 2001; Rousseau et al., 2002; Bao et al., 2019). This neuroendocrine pathway is acting as a seasonal on/off switch of the gonadotropic and metabolic axes.

Although these seasonal responses are well conserved among individuals and species, they may be threatened by new environmental constraints emerging from modern lifestyles, such as artificial light and chemical pollution. Notably, ubiquitous exposure to chemicals with endocrine-disrupting activities, called endocrine-disrupting chemicals (EDCs), may impact the seasonal neuroendocrine mechanisms, an issue still not investigated in seasonal mammals (Moralia et al., 2022). Yet, bisphenol A (BPA), a well-described EDCs massively used by the plastic and resin industries, displays oestrogenic (Kuiper et al., 1998; Kitamura et al., 2005; Delfosse et al., 2012), anti-androgenic (Wang et al., 2017), and anti-thyroid hormone activities (Moriyama et al., 2002) which could alter the complex neuroendocrine control of seasonal physiology. Moreover, BPA has been reported to modify the activity of kisspeptin, RFRP-3 (Lopez-Rodriguez et al., 2021), and POMC neurons (Desai et al., 2018; Salehi et al., 2019) that are involved in the seasonal synchronization of the gonadotropic and metabolic axes.

In this study, we tested the hypothesis that BPA exposure impacts the reproductive and metabolic adaptations to photoperiodic changes in Djungarian hamsters, with putative sex differences. Adult male and female Djungarian hamsters were thus exposed to various doses of BPA during photoperiodic transitions (either from a LP to SP or from a SP to LP) to encompass the entire period of seasonal adaptation. Weekly analyses were conducted on body weight, food intake, and pelage changes. At the end of photoperiodic adaptations (10 weeks), the animals were evaluated for reproductive organ weights, blood glucose levels, circulating sex steroid hormone levels, and gene or protein expression of key photoperiodic pathway regulators, including hypophysial and hypothalamic TSHβ, Dio2, Dio3, kisspeptin, RFRP, POMC, and somatostatin.

2. Material and methods

2.1. Animals

This project involved sexually mature (6-month-old on average)

male and female Djungarian hamsters, born and raised in the Chronobiotron animal facility (UMS 3415, Strasbourg, France) at a temperature of 22 ± 1 °C under LP (16 h light/8 h dark; with day light intensity measured at 350 lux using a digital light meter (Mannix)) condition. Hamsters were grouped by 3 or 4 in type II L cages, enriched with nest cottons, wooden sticks, and a stainless-steel tunnel, with *ad libitum* access to food and water. To avoid as much as possible environmental BPA contamination, care was taken to place animals in polyphenylsulfone cages equipped with glass drinking bottles. They were fed with a reconstituted low phytoestrogen diet (7011 P, Altromin International) prepared using glassware, stainless steel, and polypropylene tubes. All animal experiments were approved by the local ethical committee on animal experimentation (CREMEAS), in accordance with the French ministry of Higher Education and Scientific Research (authorization #22595–2019102317455140).

2.2. Experimental design

Animals were first adapted to eat the reconstituted low phytoestrogen diet for at least 6 weeks. From the start of both experimental protocols (experiment 1: transfer from LP to SP (8h light/16 h dark) or experiment 2: transfer from SP to LP), hamsters were fed with the reconstituted low phytoestrogen diet containing either 0.02% ethanol (control animals with BPA solvent) or BPA (#239658, Sigma Aldrich) adjusted to a dose of 5, 50, or 500 μg/kg of body weight/day (BPA/kg/day) in 0.02% ethanol. For each group of animals, BPA quantity added to the reconstituted food was adjusted each week according to the average body weight and amount of food intake measured in the previous week. Our estimation of BPA dosage was based on average consumption calculated from total food intake by three to four hamsters per cage (required to keep hamster sociability). This method likely introduced variability in exposure levels, as evidenced by the fluctuations in plasmatic measurements of BPA-glucuronide (Fig. 1C). However, this variability may actually better reflect real-world exposure conditions, as in natural populations, not all animals are exposed to the same levels of EDCs. As to our knowledge, limited measurements of BPA concentrations in terrestrial wildlife are reported, the doses were selected based on regulatory guidelines, intended to reflect "safe" levels of exposure. Specifically, the lowest dose of 5 μg/kg/day was chosen to be close to the tolerable daily intake of 4 μg/kg/day established from 2015 to 2023. The highest dose of 500 μg/kg/day, while not environmentally relevant, is considered as 10 times below the No Observed Adverse Effect Level (NOAEL) of 5 mg/kg bw/day established in rodents. The intermediate dose of 50 μg/kg/day was added (when possible) to assess potential non-monotonic effects.

Experiment 1 assessed the effects of BPA exposure on the reproductive and metabolic integration of SP in male and female Djungarian hamsters. Hamsters raised in LP were transferred within one day to SP for a duration of 10 weeks and fed with a diet containing either BPA solvent (vehicle group), or different doses of BPA (5, 50, or 500 μg of BPA/kg/day, n = 5 to 8 per sex and experimental group, Fig. 1A). Another group of hamsters (n = 8 to 10 per sex) was maintained in LP conditions and fed only the vehicle diet (0.02% ethanol) to serve as a reference group for active reproduction and high metabolic activity. During the 10-week SP adaptation, hamster's food intake, body weight, and fur color were monitored, and at the end of this experiment, they were sacrificed as explained below for further analyses.

Experiment 2 assessed the effects of BPA exposure on the male and female Djungarian hamster's reproductive and metabolic reactivation induced by a switchback from SP to LP (called swLP). Hamsters were first adapted to SP for 11 weeks, and animals exhibiting the expected reproductive inactivation and body weight loss were transferred within one day in LP conditions for a duration of 10 weeks and fed with a diet containing either BPA solvent (vehicle group), or different doses of BPA (5, or 500 μg of BPA/kg/day, n = 7 to 12 per sex and experimental group, Fig. 1B). During the LP adaptation, hamster's food intake, body weight, and fur color were monitored. During the initial SP adaptation, a significant increase in aggressive behavior among male hamsters, and to a lesser extend in females (as previously reported by Jasnow et al., 2000), led to considerable mortality. As a result, we were unable to evaluate the effects of the three initial selected BPA doses (5, 50, or 500 μg of BPA/kg/day), and decided to exclude the 50 μg/kg/day dose from the study. The lowest dose of 5 μg/kg/day was kept because it reflects a regulatory-relevant exposure level, close to the tolerable daily intake

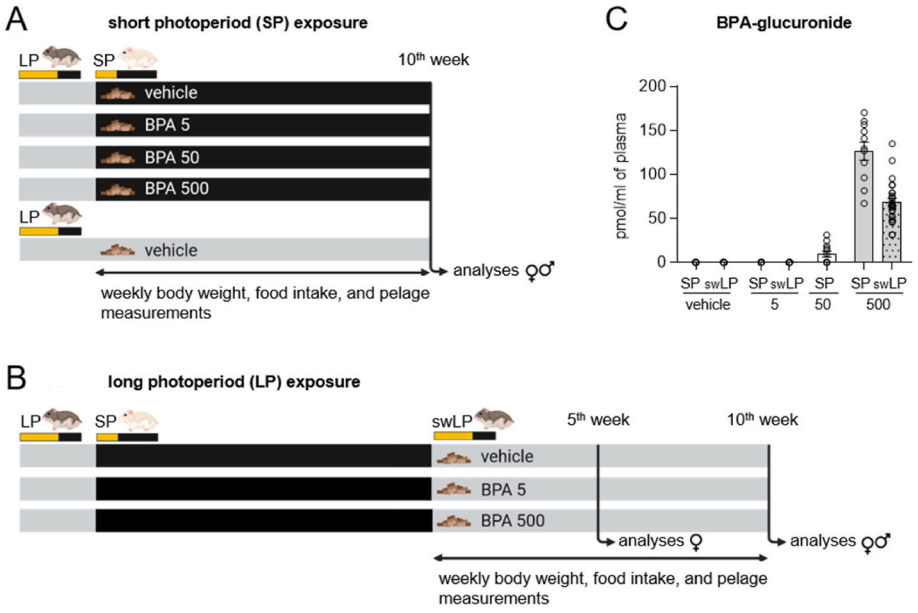

Fig. 1. Paradigms of exposure to BPA during photoperiodic transfers and plasmatic BPA-glucuronide concentrations. Female and male Djungarian hamsters were exposed to the vehicle (0.02% ethanol) or BPA at 5 (BPA 5), 50 (BPA 50), or 500 (BPA 500) μg/kg/day incorporated in food for 10 weeks of a long (LP) to short (SP) photoperiod transfer (**A**) or a SP to LP transfer (swLP) (**B**). Body weight, food intake, and pelage score were monitored each week, and various tissues were sampled at the end of the 10-week experiment (except for an additional group of females sampled 5 weeks after the SP to LP transfer). The BPA metabolite, BPA-glucuronide, was measured in the plasma of each animal to confirm the effectiveness of the administration route (**C**).

established by the EFSA at the time (4 μg/kg/day). This rearrangement led to extra females, which were used to make an intermediate sacrifice point at 5 weeks of exposure, allowing us to gather additional longitudinal data. Consequently, half of the females were sacrificed after 5 weeks, and the other half 10 weeks after the LP transfer. All males were sacrificed 10 weeks after the LP transfer.

Of note and as already reported (Gorman and Zucker, 1997), some individuals maintained a LP-like reproductive and metabolic phenotype after the SP transfer, as they did not exhibit the expected body weight loss, gonadal atrophy, or fur whitening. In our experiment, and according to the exclusion criteria established in the literature (Butler et al., 2007; Greives et al., 2008), 5 hamsters out of 148 were identified as photoperiodic non-responders and were thus removed from all the analyses.

2.3. Body weight, food intake and fur color analyses

Individual hamster's body weight was measured once a week. A non-linear regression model was used to determine the timing and speed of the photoperiodic changes in body weight, as described before (Butler et al., 2007). For each hamster, either a SP dose-response inhibition equation (experiment 1) or a LP dose-response activation equation (experiment 2) was fitted to the normalized body weight values (mean $R^2 = 0.91$ for experiment 1; mean $R^2 = 0.85$ for experiment 2) to determine acceleration in body weight change (i.e. the point of inflection of the body weight loss or body weight gain curves), and Hill slope (Motulsky and Christopoulos, 2003). For experiment 1, transition to the SP body weight phenotype was defined as the point of zero acceleration in the period of body weight loss, i.e. the point of inflection in the body weight loss curve. For experiment 2, transition to the LP body weight phenotype was defined as the point of maximum acceleration in the period of body weight gain, i.e. the point of inflection in the body weight gain curve. Speeds of body weight loss or gain were estimated from Hill slopes.

Food intake was measured once a week per cage of 3–4 hamsters. Cumulative feeding efficiency ratios were determined by dividing cumulative individual body weight gain or loss by the average cumulative food intake of each cage, over all weeks of experimental treatment.

The change in fur coloration and density was scored weekly using an index described by Figala et al. (1973). The score ranged from 1 for grey summer fur to 5 for white winter fur.

2.4. Tissue sampling

At the end of experiments, all hamsters were sacrificed during the early light phase (between 1 and 4 h after lights on) with the females being sacrificed in diestrus to reduce hormonal bias. Each hamster was deeply anesthetized (40 mg/kg of tiletamine/zolazepam (Zoletil®50) and 10 mg/kg of xylazine (Paxman)), then blood was collected by heart puncture, and the body was fixed by a transcardiac perfusion with phosphate buffered saline (PBS) followed by 4% paraformaldehyde-lysine-periodate (PLP) fixative. After perfusion, reproductive organs (uterus with ovaries for females and pairs of testes for males) were directly dissected and weighed. Brains were extracted and conserved in the PLP solution for 12 h before dehydration and polyethylene glycol embedding (Klosen et al., 1993). Each brain was cut into 10 μm-thin coronal sections from the preoptic area to the mammillary bodies, and 10 sets of 16 serial sections covering the full extent of this hypothalamic area were mounted on Superfrost Plus glass slides, selecting one out of every 10 sections (consecutive sections being 100 μm apart). Thus, each glass slide contained 16 sections spanning the hypothalamus of one animal. Slides were dried 20 min at 50 °C and stored at −80 °C until use for neuroanatomical analyses.

2.5. Measurements of circulating BPA metabolite, sex steroids, glucose, and metabolic hormones

2.5.1. Plasma testosterone and BPA-glucuronide

Plasma testosterone and BPA-glucuronide were analyzed by LC/MS-MS. Briefly, 15 μl of internal standards (3.3 μM -[^{2}H4]-testosterone + 13.3 μM [^{13}C12]-BPA-glucuronide) and 450 μl of acetonitrile (ACN) were mixed to 150 μl of plasma sample. After centrifugation at 20,000×*g* for 30 min at 4 °C, the supernatant was collected, dried under vacuum, and resuspended in 30 μl of 40% ACN/0.1% formic acid (v/v), and centrifuged at 20,000×*g* for 20 min at 4 °C. The final supernatant was divided in two samples of 15 μl for testosterone and 15 μl for BPA-glucuronide analyses. For testosterone assay only, the 15 μl supernatant was dried under vacuum and resuspended in 15 μl of Amplifex Keto Reagent Kit solution (Waters) for 1 h at room temperature to enhance testosterone LC/MS-MS signal, and finally centrifuged at 20,000×*g* for 10 min at 4 °C. Analyses were conducted on 4 μl of both samples with a Dionex Ultimate 3000 HPLC system coupled with a triple quadrupole Endura mass spectrometer (Thermo Electron) in the positive (testosterone) or negative mode (BPA-glucuronide). Separation of the testosterone and BPA-glucuronide were done at 40 °C on a Zorbax column with a gradient of ACN (see Supplementary Tables S1 and S2). The identification of the compounds was based on precursor ions, selective fragment ions (i.e. daughter ions) and retention times obtained for testosterone, BPA-glucuronide, and their internal standards. Quantification was done by the calculation of the peak area ratio between the daughter ion used for quantification of testosterone or BPA-glucuronide and their internal standards (Supplementary Tables S1 and S2). The limits of detection (LOD) were defined as the lowest detectable amount of analyte with a signal-to-noise (S/N) ratio greater than 3. The LOD for BPA-glucuronide and keto-derived testosterone were 7 fmol and 2.4 fmol per injection, respectively. The limit of quantification (LOQ) was defined as the lowest detectable amount of analyte with a signal-to-noise (S/N) ratio greater than 10. The LOQ for BPA-glucuronide and keto-derived testosterone were 27 fmol and 9.8 fmol per injection, respectively. All amounts of compounds measured in samples fit within the standard curve limits, from 0 to 55 pmol per injection for BPA-glucuronide and from 0 to 20 pmol per injection for keto-derived testosterone.

The measurement of plasma BPA-glucuronide, the major metabolite of BPA, allowed to confirm that BPA exposure induced relevant concentrations of BPA in the organism of exposed animals, and to verify that control animals were exposed to minimal levels of environmental BPA (Fig. 1C). The absence of BPA-glucuronide detection in the plasma of the 5 μg BPA/kg/day-exposed animals is due to the fact that values are below the LOD due to a limit of sensitivity of our assay.

2.5.2. Plasma estradiol

Plasma estradiol was measured after extraction in methanol according to the Estradiol ELISA Kit (#501890, Cayman Chemical). Intra- and inter-assay variations were <5%, and the assay displayed a sensitivity of 20 pg/ml.

2.5.3. Plasma insulin

Plasma insulin was measured in 20 times diluted plasma samples using the Hamster Insulin ELISA Kit (#90336, CrystalChem). Intra-assay variations were <10% and inter-assay variations were <15%. The assay has a limit of sensitivity of 0.05 ng/ml.

2.5.4. Blood glucose

Blood glucose levels were measured immediately in one drop of blood after the heart puncture with the Blood Glucose System Accu-Chek Performa (Roche Diagnostics).

2.6. Neuroanatomical analyses

For each staining, all slides of all animals belonging to the same experimental protocol were processed together. Fig. 2 illustrates the neuroanatomical localisation of the investigated genes and protein.

2.6.1. Non-radioactive in situ hybridization

Non-radioactive *in situ* hybridization was performed on fixed hypothalamic sections using antisens riboprobes transcribed from 1 μg linearized plasmid DNA with digoxigenin or fluorescein-labelled nucleotides. Djungarian hamster's *Dio2*, *Dio3* and *Rfrp* probes were used, as well as rat *TSHβ*, *Pomc*, and *somatostatin* probes that were all previously validated on Djungarian hamster tissue (Milesi et al., 2017; Cázarez-Márquez et al., 2019).

Briefly, the sections were post-fixed for 10 min with 4% formalin in phosphate buffer (PB), rinsed in PBS, digested for 30 min at 37 °C with 0.5–1 μg/ml of proteinase K (Roche), quickly rinsed with cold PBS, post-fixed with 2% formalin in PB on ice, rinsed in PBS + 0.01% diethylpyrocarbonate (DEPC), acetylated twice for 10 min in 100 mM triethanolamine + 0.25% acetic anhydride, washed in PBS + 0.01% DEPC and equilibrated in 5X saline sodium citrate (SSC) + 0.05% Tween 20 + 0.01% DEPC. The sections were hybridized with 200 ng/ml denaturated riboprobes diluted in 50% formamide + 5X SSC + 5X Denhardt's solution +1 mg/ml of salmon DNA +0.1% Tween 20 + 0.04% DEPC for 40 h at 54–60 °C. After hybridization, sections were washed in SSC 5X + 0.05% Tween 20 and then in SSC 0.1X + 0.05% Tween 20 at 72 °C to remove non-specific hybrids. Sections were washed in A-DIG buffer + 0.05% Tween 20, blocked for at least 1 h with blocking buffer (Roche), and incubated overnight with the alkaline phosphatase (AP) coupled anti-digoxigenin or anti-fluorescein antibody (Roche) at respectively 1/5000 or 1/1000 in blocking buffer. Sections were rinsed in A-DIG buffer + 0.05% Tween 20 and equilibrated in AP 1X buffer. AP activity was detected either with a solution of nitro blue tetrazolium (Roche)/bromo-chloro-indolyl phosphate (Thermo Scientific) in AP buffer or using Naphthol AS-MX phosphate and Fast Red (Sigma-Aldrich) as substrate for 2–24 h depending on the probes. Reactions were stopped with tap water before the staining intensity reached saturation. Slides were pre-mounted with CrystalMount (Sigma-Aldrich), dipped in toluene, and mounted with a coverslip and Eukitt (Sigma-Aldrich).

2.6.2. Immunohistochemistry

Because *Kiss1* mRNA expression in the ARC is difficult to quantify in Djungarian hamsters, we used immunolabelling to detect ARC kisspeptin (Milesi et al., 2017). Brain sections were treated for antigen reactivation with citrate buffer at 95 °C for 2 h, cooled down to room temperature and rinsed with tris-buffered saline (TBS). Slides were pretreated with a blocking reagent (TBS + 3% powder milk + 0.02% NaN_3) for at least 1 h before being incubated overnight with the primary rabbit kisspeptin antibody (JLV-1 antiserum against the rat kisspeptin-52, Mikkelsen and Simonneaux, 2009) diluted at 1/1500 in TBS + 0.05% Tween 20 + 1% donkey serum + 0.02% NaN_3. Sections were rinsed in TBS + 0.02% Tween 20, incubated for 1 h with a biotinylated secondary antibody (donkey anti-rabbit (Jackson) diluted at 1/2000 in TBS + 0.02% Tween 20 + 1% donkey serum), rinsed in TBS + 0.02% Tween 20, and incubated with a neutravidin-horseradish peroxidase (HRP, Thermo Scientific) solution at 1/ 2000 in TBS + 0.02% Tween 20 + 0.2% cold water fish gelatin for 1 h. Sections were washed in TBS + 0.02% Tween 20, equilibrated in tris-imidazole buffer (TBI), and HRP activity was detected with a solution of 1% diaminobenzidine (Acros Organics) + 0.003% H_2O_2 in TBI. Sections were dehydrated in alcohol baths and coverslipped with Eukitt.

2.6.3. Image analysis

For *somatostatin*, *Pomc*, *TSHβ*, and *Dio3* staining obtained from

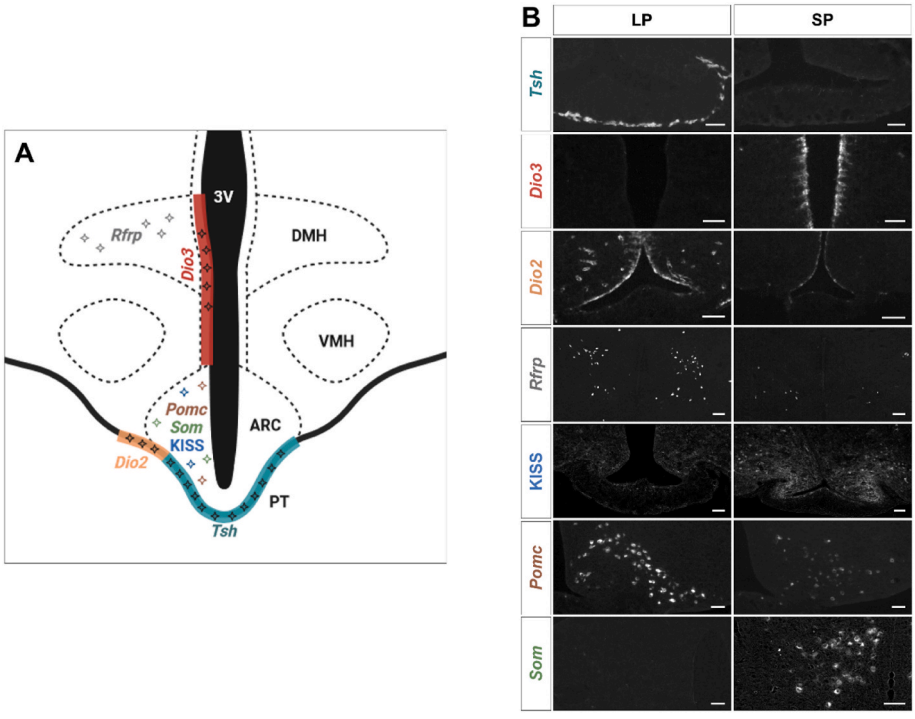

Fig. 2. Representation of the anatomical location of the photoperiodic genes and proteins analyzed using histological techniques. (A) The mRNA or protein expression of *Pomc*, *somatostatin* (*Som*), and kisspeptin (KISS) was quantified in the arcuate nucleus (ARC), *Rfrp* in the dorsomedial hypothalamus (DMH). *Dio2* analysis was performed on the tanycyte endfeets in the tuberoinfundibular sulcus, while *Dio3* was analyzed alongside the third ventricle (3V) in the α-tanycytes body cells. *TSHβ* expression was analyzed alongside the *pars tuberalis* (PT). **(B)** Representative photomicrographs of *TSHβ, Rfrp, Dio3, somatostatin, Pomc in situ* hybridization, and kisspeptin IHC for female controls adapted to a long photoperiod (LP) or a short photoperiod (SP) (scale bar, 50 μm). VMH = ventromedial hypothalamus.

experiment 1, micrographs were taken at 10 X 0.63 objective by a DP 50 digital camera (Olympus) attached to a DMRB microscope (Leica). A background image without tissue was taken for each slide and subtracted from sampled images. The images were analyzed using ImageJ software (Rasband, U.S. National Institute of Health). Mean grey value pixels of *somatostatin* and *Pomc* labelling in neurons were measured using a fixed-size circle overlaid on at least 50 neurons per animal, as described before (Klosen et al., 2013; Cázarez-Márquez et al., 2019). As *somatostatin* mRNA expression exhibits photoperiodic variations only in the caudal part of the ARC (Herwig et al., 2012), *somatostatin* neuron labelling intensity was measured only in 3–4 images/animal of this region. *Pomc* neuron labelling intensity was measured in 6–7 images/animal covering the full extent of the ARC. *TSHβ* integrated density was measured in 3–4 images/animal using a 65-μm wide line tool covering the *pars tuberalis*. For *Dio3*, we used standardized thresholding to select and measure integrated density using a 150-μm wide line tool in Dio3-stained areas (in α tanycytes) of 3 thresholded images/animal. *Rfrp* labelled neurons were observed with an optic microscope equipped with a camera, counted manually, and normalized by the number of sections counted. When mouting, we included sections that did not contained *Rfrp* labelling rostrally and caudally to ensure a total count of the number of hypothalamic *Rfrp* expressing neurons.

For kisspeptin staining and all stainings obtained from the experiment 2, slides were scanned with the Nanozoomer 2.0 HT (Hamamatsu) using the program NDP.scan. The imageJ analyses for *Dio2*, *TSHβ*, and *somatostatin* stainings was the same as described for the experiment 1. In order to improve consistency of counts and to detect all labelled cells when the number of cells is important, as it is for kisspeptin, *Pomc* and *Rfrp* expressing neurons, an automatic cell detection was performed using QuPath software (v0.4.0, Bankhead et al., 2017). We used the StarDist deep-learning-based method of cell detection to count kisspeptin labelled cells, based on the pre-trained model 'dsb2018_heavy_augment.pb' (Schmidt et al., 2018). *Pomc* and *Rfrp* labelled neurons were detected automatically based on morphological and intensity criteria, with post-checking that the detected objects were indeed labelled cells of interest.

2.7. Statistical analysis

All data are presented as mean ± SEM of n animals (from 5 to 12 according to the experimental groups) and were analyzed using GraphPad Prism 8.0.2. The appropriate sample size was determined using G*Power for a Two-Way analysis of variance (ANOVA) with repeated measures across our four experimental groups (BPA 0, BPA 5, BPA 50, and BPA 500), with 10 measurements taken weekly. For each statistical analysis, the normality assumption was checked using the Shapiro-Wilk test, and the homogeneity of variance assumption was evaluated with a homoscedasticity plot. Repeated values over time were analyzed by Two-Way ANOVA followed by Dunnett's *post hoc* tests. Mixed-model effects were used instead of Two-Way ANOVA when values were missing. For the females of the experiment 2, for each dose, the dynamic changes of mRNA/protein expression between the 5th and the 10th week were analyzed using Two-Way ANOVA with Sidak *post hoc* tests for multiple comparisons. Multiple group comparisons were analyzed using One-Way ANOVA or Kruskal-Wallis tests, followed by Dunnett's *post hoc* test. To ascertain the photoperiodic effect, comparisons between the LP- and SP- or swLP- vehicle groups were performed using t-tests. Statistical significance was set at p-value <0.05.

3. Results

3.1. Effect of oral BPA exposure on the physiological and neuroendocrine short photoperiod integration in female and male hamsters

3.1.1. Exposure to a high dose of BPA delays metabolic integration in female hamsters under short photoperiod

All female hamsters showed an expected decrease in body weight upon transfer to SP, while those kept in LP maintained stable body weight (Fig. 3A). However, females exposed to 500 μg BPA/kg/day displayed a delayed SP-induced body weight loss by more than one week compared to those exposed to the vehicle (0.02% ethanol), 5, or 50 μg of BPA/kg/day (Fig. 3B). Furthermore, females exposed to 500 μg BPA/kg/day demonstrated higher feed efficiency ratios (FER) during the initial two weeks of SP compared to the vehicle-exposed group (Fig. 3C). After 10-week SP exposure, all female hamsters lost an average of 24.6% (±1.1%) of their body weight whether exposed or not to BPA (Fig. 3B). The glucose/insulin (G/I) ratio (Table 1), an indicator of insulin sensitivity, was similar among all female groups. Fur color whitening was also delayed in females exposed to 500 μg BPA/kg/day as compared to the vehicle-exposed group, although all females exhibited a similar white fur color 10 weeks after the SP transfer (Fig. 3D).

At the end of the 10-week SP exposure, all female hamsters exhibited the expected decrease in the relative uterine and ovary weight, with no significant effect of BPA exposure on the final regression of the reproductive organs (Table 1). Circulating concentrations of estradiol were similar among all female hamster groups.

The selected genes or proteins known to adapt reproductive and metabolic activities to photoperiodic changes showed the expected SP-induced decrease (*TSHβ*, *Rfrp*) and increase (*Dio3,* kisspeptin, *somatostatin*) in their expression 10 weeks after the SP transfer, compared to LP hamsters (Fig. 3E). However, exposure to 500 μg BPA/kg/day abolished the SP-induced increase in ARC somatostatin. The photoperiodic changes in the other investigated genes or protein were not altered by BPA exposure.

3.1.2. Exposure to a low dose of BPA accelerates metabolic integration in male hamsters under short photoperiod

All male hamsters switched to SP gradually reduced their body weight (Fig. 4A). However, the use of a non-linear regression model indicates that the onset of the SP body weight phenotype appeared one week earlier in hamsters exposed to 5 μg BPA/kg/day compared to hamsters exposed to vehicle, 50, or 500 μg BPA/kg/day (Fig. 4B). The feed efficiency ratio was also lower in male hamsters exposed to 5 μg BPA/kg/day compared to the vehicle-exposed group 5 weeks after the SP transfer (Fig. 4C), and the G/I ratio was higher for the 5 μg BPA/kg/day-exposed group compared to the other experimental groups (Table 1). The speed of the SP-induced body weight loss was similar among all groups of male hamsters, and all lost an average of 23.9% (± 1.4%) of their body weight 10 weeks after the SP transfer, whether exposed or not to BPA (Fig. 4B).The pelage bleaching was significantly advanced in males exposed to 5 and 50 μg BPA/kg/day at the 8th week of SP, but after 10 weeks of SP exposure, all hamsters exhibited the same white and fluffy fur, whether exposed or not to BPA (Fig. 4D).

After 10 weeks of SP, all male hamsters had low testis weight, compared to hamsters kept in LP, and BPA exposure did not affect the final SP-induced regression of reproductive organs (Table 1). Plasma testosterone was only detectable in male hamsters kept in LP and was below the limit of detection for all SP-adapted groups.

As for female hamsters, all of the selected reproductive and metabolic genes or proteins showed the expected SP-induced changes compared to the LP control group, with the exception of *Pomc* mRNA expression, which remained at the same levels as the LP control group. Exposure to various doses of BPA did not modify the final SP-induced changes in these gene and protein expressions (Fig. 4E).

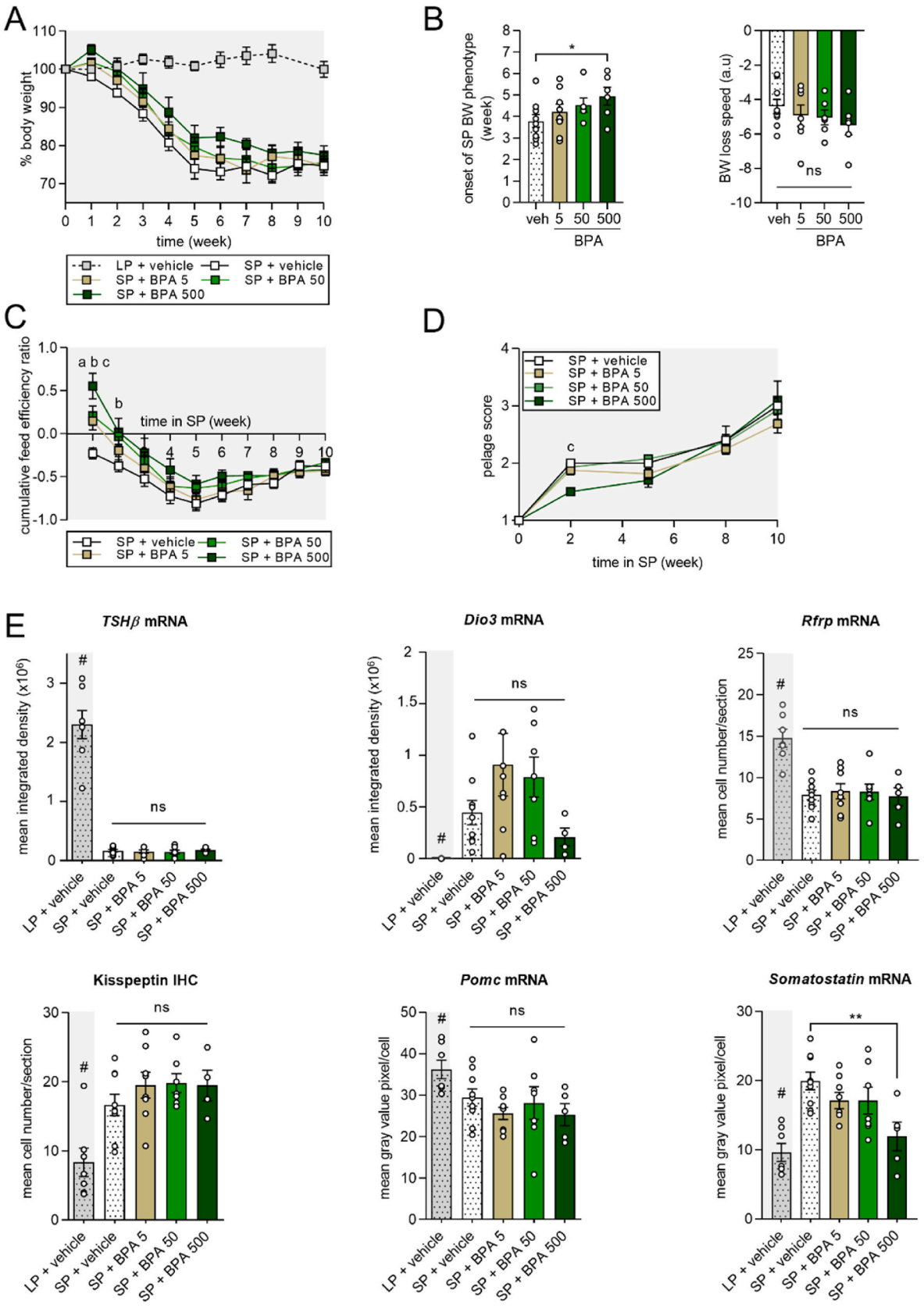

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Fig. 3. Effects of BPA exposure on body weight, food intake, pelage whitening, and various photoperiodic genes/proteins in female Djungarian hamsters transferred from long to short photoperiod. Female Djungarian hamsters were either kept in long photoperiod (LP) and fed with pellet food containing 0.02% ethanol (LP + vehicle; n = 8) or transferred in short photoperiod (SP) for 10 weeks and fed with pellet food containing 0.02% ethanol (SP + vehicle; n = 9), or containing 5 (SP + BPA 5; n = 8), 50 (SP + BPA 50; n = 7), or 500 (SP + BPA 500; n = 6) μg BPA/kg/day. **(A)** Body weight (BW) expressed in percentage of the LP value (before the SP transfer) during the 10 weeks of photoperiodic treatment. **(B)** Time in weeks to reach the SP body weight phenotype (point of inflection calculated from a non-linear regression model fitted to individual body weight raw data) and speed of SP-induced body weight loss (slope of the non-linear regression). **(C)** Cumulative feed efficiency ratio over 10 weeks of SP. **(D)** Pelage score (from 1 being a LP-adapted grey color to 5 being a SP-adapted white color) over 10 weeks of SP. **(E)** Expression of *pars tuberalis TSHβ*, and hypothalamic *Dio3*, *Rfrp*, *Pomc*, and *somatostatin* gene and kisspeptin protein in constant LP (grey bar) or after 10 weeks of SP. Values are given as mean ± SEM (n = 6 to 9 according to experimental groups). Statistical significance: for repeated values over time, *a*, $p < 0.05$ between SP + BPA 5 vs SP + vehicle, *b*, $p < 0.05$ between SP + BPA 50 vs SP + vehicle, *c*, $p < 0.05$ between SP + BPA 500 vs SP + vehicle; for multiple comparisons between groups, *, $p < 0.05$ vs SP + vehicle; **, $p < 0.01$ vs SP + vehicle; to ascertain the photoperiodic effects on gene/protein expression, comparisons were also made between the LP + vehicle and SP + vehicle groups (# when $p < 0.05$). ns = no significance; veh = vehicle.

Table 1
Effects of BPA exposure on reproductive and metabolic parameters in female and male Djungarian hamsters transferred from long to short photoperiod. Hamsters were orally exposed (through food) to 0.02% ethanol (SP + vehicle; n = 8–9) or to 5 (SP + BPA 5; n = 7–8), 50 (SP + BPA 50; n = 6–7), or 500 (SP + BPA 500; n = 5–6) μg of BPA/kg/day during a 10-week transfer from a long (LP) to a short photoperiod (SP). A control group of hamsters was kept under LP (LP + vehicle; n = 8–10). Values are indicated as mean ± SEM. Statistical significance: #, $p < 0.05$ between LP + vehicle vs SP + vehicle, *a*, $p < 0.05$ between SP + BPA 5 vs SP + vehicle. LOD = below the limit of detection.

photoperiod/dose	females					males				
	LP + vehicle (n = 8)	SP + vehicle) (n = 8)	SP + BPA 5 (n = 8)	SP + BPA 50 (n = 7)	SP + BPA 500 (n = 5)	LP + vehicle (n = 10)	SP + vehicle (n = 8)	SP + BPA 5 (n = 7)	SP + BPA 50 (n = 6)	SP + BPA 500 (n = 6)
relative reproductive organ weight (mg/g of body weight)	2.5 ± 0.2#	1.7 ± 0.1	2.0 ± 0.2	1.8 ± 0.2	1.9 ± 0.2	20.3 ± 1.8#	1.8 ± 0.3	1.6 ± 0.1	1.6 ± 0.2	1.5 ± 0.1
estradiol (pg/ml of plasma)	283 ± 63	232 ± 115	337 ± 125	162 ± 46	147 ± 45					
testosterone (pg/ml of plasma)						35.8 ± 15.7	LOD	LOD	LOD	LOD
glucose/insulin ratio	19.4 ± 4.1	24.4 ± 2.9	27.2 ± 4.2	25.7 ± 5.3	24.8 ± 3.4	14.4 ± 3.8	23.5 ± 5.4	49.2 ± 10.2[a]	40.1 ± 11.3	34.4 ± 5.2

3.2. Effect of oral BPA exposure on the physiological and neuroendocrine integration of long photoperiod in male and female hamsters

After a prior full adaptation to 11 weeks of SP exposure, male and female hamsters were switched back to LP (swLP) with or without oral exposure to BPA for a subsequent 10-week period. The 50 μg/kg/day BPA dose was excluded in this second study based on observations from the previous experiment, where the 5 and 500 μg/kg/day doses showed more pronounced physiological and neuroendocrine changes. Further, as the previous BPA exposure was found to alter the kinetics of some aspects of photoperiodic adaptation, intermediate groups of hamsters were added to be sampled 5 weeks after the swLP transfer. However, due to a large number of male hamster deaths, only female hamsters were available for sampling at this intermediate stage.

3.2.1. BPA exposure induces a faster metabolic and reproductive adaptation to long photoperiod in female hamsters

All females transferred back to LP conditions gradually regained body weight (Fig. 5A), although females exposed to 5 and 500 μg BPA/kg/day experienced a faster body weight gain (Fig. 5B) and a higher increase in feed efficiency ratios in the initial two weeks of swLP (Fig. 5C) compared to vehicle-exposed females. After 10 weeks in swLP, all females gained an average of +23.1% (± 4.7%) body weight, regardless of BPA exposure (Fig. 5A), and blood glucose concentrations were comparable across all experimental groups (Table 2). Throughout the 10 weeks of swLP, female hamster coats remained whitish, with no observable differences among groups (Fig. 5D).

Five weeks after swLP, BPA-exposed females displayed significantly different relative uterine and ovarian weight compared to vehicle-exposed females (Table 2). Those exposed to 5 μg BPA/kg/day tended to have higher reproductive organ weight, while those exposed to 500 μg BPA/kg/day tended to have lower weight (One-way ANOVA, $p < 0.05$, but no significant *post hoc* tests). After 10 weeks of swLP, all female groups reached a fully active reproductive state, as indicated by high reproductive organ weight and circulating estradiol levels (Table 2).

The comparison of mRNA/protein levels of most photoperiodic, metabolic, and reproductive genes and protein between the mid (5 weeks) and end (10 weeks) of swLP revealed the dynamic change during swLP integration (Fig. 5E). Levels of the majority of investigated proteins/genes in 10-week swLP vehicle-treated females were similar to those of females maintained constantly in LP. However, exposure to 5 μg BPA/kg/day was associated with a significant decrease in kisspeptin level between the mid and end of swLP, suggesting a faster kisspeptin downregulation induced by the low BPA dose. Furthermore, in contrast to vehicle-treated hamsters, exposure to both doses of BPA abolished *Pomc* mRNA increase between the 5th and the 10th week of swLP, and exposure to 5 μg BPA/kg/day induced a lower *Pomc* mRNA expression at the 10th week of swLP, suggesting that BPA delayed the LP-induced *Pomc* upregulation.

3.2.2. BPA exposure during the long photoperiod adaptation of male hamsters alters the expression of photoperiodic genes but does not induce metabolic and reproductive effects

All male hamsters transferred from an SP to an LP condition gradually regained body weight, (approximatively +18.1 ± 3.4 % of initial (SP) body weight; Fig. 6A), and increased their feed efficiency ratios (Fig. 6B) without any significant impact of BPA exposure. Similarly, all hamster groups exhibited comparable blood glucose concentrations, independently of BPA exposure (Table 2). Throughout the 10 weeks of swLP, male hamster coats remained whitish, regardless of BPA exposure (Fig. 6C).

After 10 weeks of swLP, male hamsters had regained high relative testis weights, as well as circulating testosterone concentrations (Table 2) characteristic of sexually active LP-adapted animals. BPA exposure did not modify these reproductive indexes.

Expression levels of the photoperiodic genes *TSHβ*, Dio2, and *Rfrp* 10

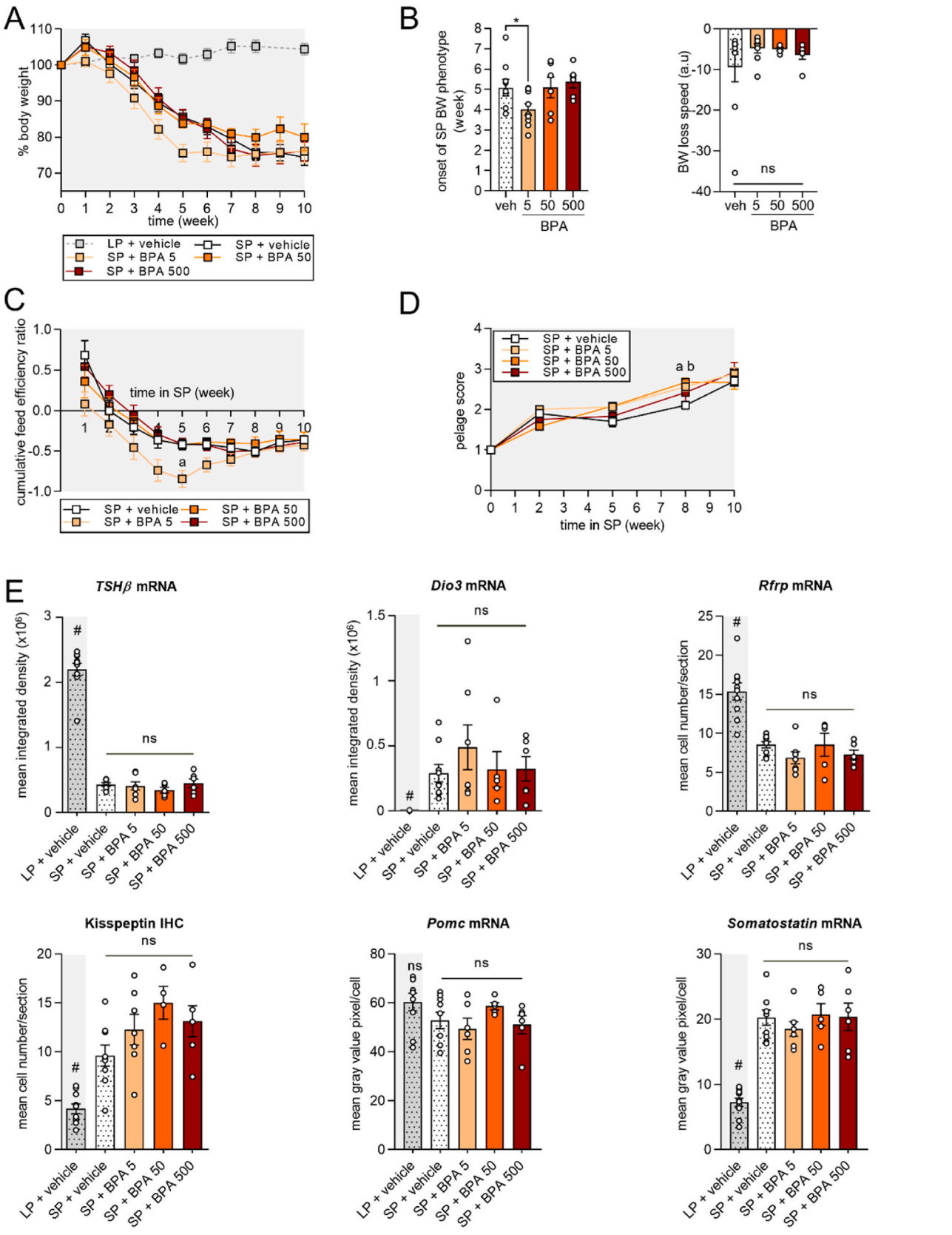

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Fig. 4. Effects of BPA exposure on body weight, food intake, pelage whitening, and various photoperiodic genes/proteins in male Djungarian hamsters transferred from long to short photoperiod. Male Djungarian hamsters were either kept in long photoperiod (LP) and fed with pellet food containing 0.02% ethanol (LP + vehicle; n = 10), or transferred in short-photoperiod (SP) and fed with a diet containing 0.02% ethanol (SP + vehicle; n = 9), or containing 5 (SP + BPA 5; n = 8), 50 (SP + BPA 50; n = 6), or 500 μg BPA/kg/day (SP + BPA 500; n = 6). **(A)** Body weight (BW) expressed in percentage of the LP value (before the SP transfer) during the 10 weeks of photoperiodic treatment. **(B)** Time in weeks to reach the SP body weight phenotype (point of inflection calculated from a non-linear regression model fitted to individual body weight raw data) and speed of SP-induced body weight loss (slope of the non-linear regression). **(C)** Cumulative feed efficiency ratio over 10 weeks of SP. **(D)** Pelage score (from 1 being a LP-adapted grey color to 5 being a SP-adapted white color) over 10 weeks of SP. **(E)** Expression of *pars tuberalis TSHβ*, and hypothalamic *Dio3*, *Rfrp*, *Pomc*, and *somatostatin* gene and kisspeptin protein after 10 weeks of SP or constant LP (grey bar). Values are given as mean ± SEM (n = 6 to 10 according to experimental groups). Statistical significance: for repeated values over time, *a*, $p < 0.05$ between SP + BPA 5 vs SP + vehicle, *b*, $p < 0.05$ between SP + BPA 50 vs SP + vehicle; for multiple comparisons between groups, *, $p < 0.05$ vs SP + vehicle; to ascertain the photoperiodic effects on gene/protein expression, comparisons were also made between the LP + vehicle and SP + vehicle groups (# when $p < 0.05$). ns = no significance; veh = vehicle.

weeks after the switch from SP to LP did not reach values similar to those observed in hamsters kept constantly in LP, suggesting a still intermediate state at this stage (Fig. 6E). Furthermore, BPA exposure during swLP reduced the expression of *TSHβ* and *Rfrp* (at the dose of 5 μg/kg/day) and *Dio2* (at the dose of 500 μg/kg/day) (Fig. 6E). In contrast, the expression of the metabolic genes *Pomc* and *somatostatin,* and the reproductive protein kisspeptin, were not altered by either dose of BPA exposure after 10 weeks of swLP (Fig. 6E).

4. Discussion

The present study is, to the best of our knowledge, the first investigation of the impact of BPA exposure on the photoperiodic adaptation of a seasonal mammal. It demonstrates that BPA-enriched food disrupts the kinetics of Djungarian hamster's physiological adaptation to seasonal changes in photoperiod, underscoring a marked sex-dependent effect of BPA on seasonal physiology.

4.1. BPA exposure exerts a negligible effect on the integration of the photoperiodic message

Photoperiodic integration is known to involve a melatonin-driven change in *pars tuberalis* TSH action on hypothalamic thyroid hormone metabolism and RFRP-3 neurons (Dardente and Simonneaux, 2022 for review). In our study, none of the investigated doses of BPA (from 5 to 500 μg/kg/day) modified the SP-induced decrease in *TSHβ* and *Rfrp* mRNA and the increase in *Dio3* mRNA in both male and female hamsters. This suggests that, at least in the Djungarian hamster, BPA does not alter the SP melatonin signaling up to the RFRP-3 neurons. The LP-induced increase in *TSHβ, Dio2, and Rfrp* mRNA levels was not affected by BPA in female hamsters, but in males BPA exposure reduced *TSHβ, Dio2, and Rfrp* levels. This could result from BPA acting on the melatoninergic message (potentially synthesis, transport, degradation, or MT1 receptors in the *pars tuberalis*), but this is unknown, as the majority of experimental studies assessing the effects of BPA are based on melatonin-deficient mouse models or are not conducted in seasonal/photoperiodic contexts. BPA could also act directly on *pars tuberalis TSHβ* synthesis, as it has been reported to act as a blocker of TSH secretagogues in bullfrog pituitaries (Kaneko et al., 2008). However, neonatal exposure to BPA was not found to alter pituitary mRNA *TSHβ* expression in female rats (Fernandez et al., 2018). The decreased expression of *Dio2* and *Rfrp* levels observed in LP-transferred males exposed to BPA could result from the reduction in *TSHβ* expression, as TSH acts sequentially on these hypothalamic factors, or due to a direct effect. Indeed, BPA was reported to inhibit Dio2 in brown adipose tissue *in vitro* (da Silva et al., 2019). Additionally, one study showed that female rats prenatally exposed to 50 μg/kg BPA exhibit decreased *Rfrp* expression and a reduction in the number of contacts between RFRP-3 fibres and GnRH neurons, associated with advanced vaginal opening (Losa-Ward et al., 2012). Nevertheless, in our case, this BPA-induced reduction in the *TSHβ/Dio2/Rfrp* pathway in male hamsters was not associated with an obvious disruption of metabolic or reproductive responses to the LP transfer.

4.1.1. Metabolic adaptation to photoperiodic changes is altered by BPA with marked sex-specific differences

BPA exposure significantly disturbed the kinetics of the Djungarian hamster's metabolic adaptation to photoperiodic changes, revealing marked differences based on both the photoperiodic protocol and the hamster's sex. Specifically, exposure to BPA during the LP to SP transfer slowed the acquisition of the lean metabolic phenotype in females exposed to 500 μg/kg/day but accelerated it in males exposed to 5 μg/kg/day. On the other hand, exposure to 5 or 500 μg/kg/day BPA during the SP to LP transfer accelerated the acquisition of the fat metabolic phenotype in females but had no obvious metabolic effect in males.

BPA has been previously identified as an obesogenic substance in multiple *in vitro* and *in vivo* studies (Moghaddam et al., 2015; Rubin et al., 2001; Angle et al., 2013; Marmugi et al., 2012; Alonso-Magdalena et al., 2006, 2010) and its exposure has been linked to increased risks of obesity and type 2 diabetes in humans (Lang et al., 2008; Sun et al., 2014; Song et al., 2016). The observation that female Djungarian hamsters exposed to BPA show a slowdown in body weight loss and a decrease in feed efficiency during the transfer to SP, as well as an acceleration in body weight gain and an increase in feed efficiency in swLP is therefore consistent with this obesogenic effect of BPA. The SP-induced decrease in Djungarian hamster's body weight is known to depend on a TSH/T3-driven increase in ARC *somatostatin* expression (Dumbell et al., 2015; Klosen et al., 2013). Therefore, the slower rate of SP-induced body weight loss in female hamsters exposed to 500 μg/kg/day BPA could be attributed to the reduced expression of ARC *somatostatin* observed after 10 weeks of SP. These results are in line with previous studies reporting that BPA can alter the binding activities of the somatostatin subtype 3 receptors in the rat ARC (Facciolo et al., 2005), inhibit the synthesis and *in vitro* release of growth hormone (GH) via an alteration in the cellular signal transduction systems of GHRH (Katoh et al., 2004), and alter ERα receptors in a small population of ARC somatostatin neurons (Scanlan et al., 2003). The photoperiodic regulation in female Djungarian hamster's body weight is also associated with changes in *Pomc* gene expression, known to be higher in LP compared to SP (Cázarez-Márquez et al., 2019; Mercer et al., 2000; Reddy et al., 1999). Therefore, the accelerated body weight gain and improved feeding efficiency observed in female hamsters exposed to 5 μg/kg/day BPA during the SP to LP transfer may result from the observed reduction in the ARC *Pomc* expression. Previous studies have reported that BPA can disrupt *Pomc* expression, with contradictory variations depending on the study (Desai et al., 2018; Salehi et al., 2019) and induce a reduction in the number of POMC neuron projections to the paraventricular nuclei (Mackay et al., 2013). BPA effect on *Pomc* expression could also be mediated by a direct antagonistic disruption of T3, which has been shown to induce *Pomc* expression by binding to TRβ1 in POMC neurons (Bao et al., 2019).

In male Djungarian hamsters transferred from LP to SP, the acceleration of body weight loss and reduction in feed efficiency induced by exposure to 5 μg/kg/day BPA was unexpected given the recognized obesogenic effect of BPA. However, BPA exposure in humans is sometimes associated with a reduction in body mass indexes (Harley et al., 2013). No change in *Pomc* and *somatostatin* gene expression was

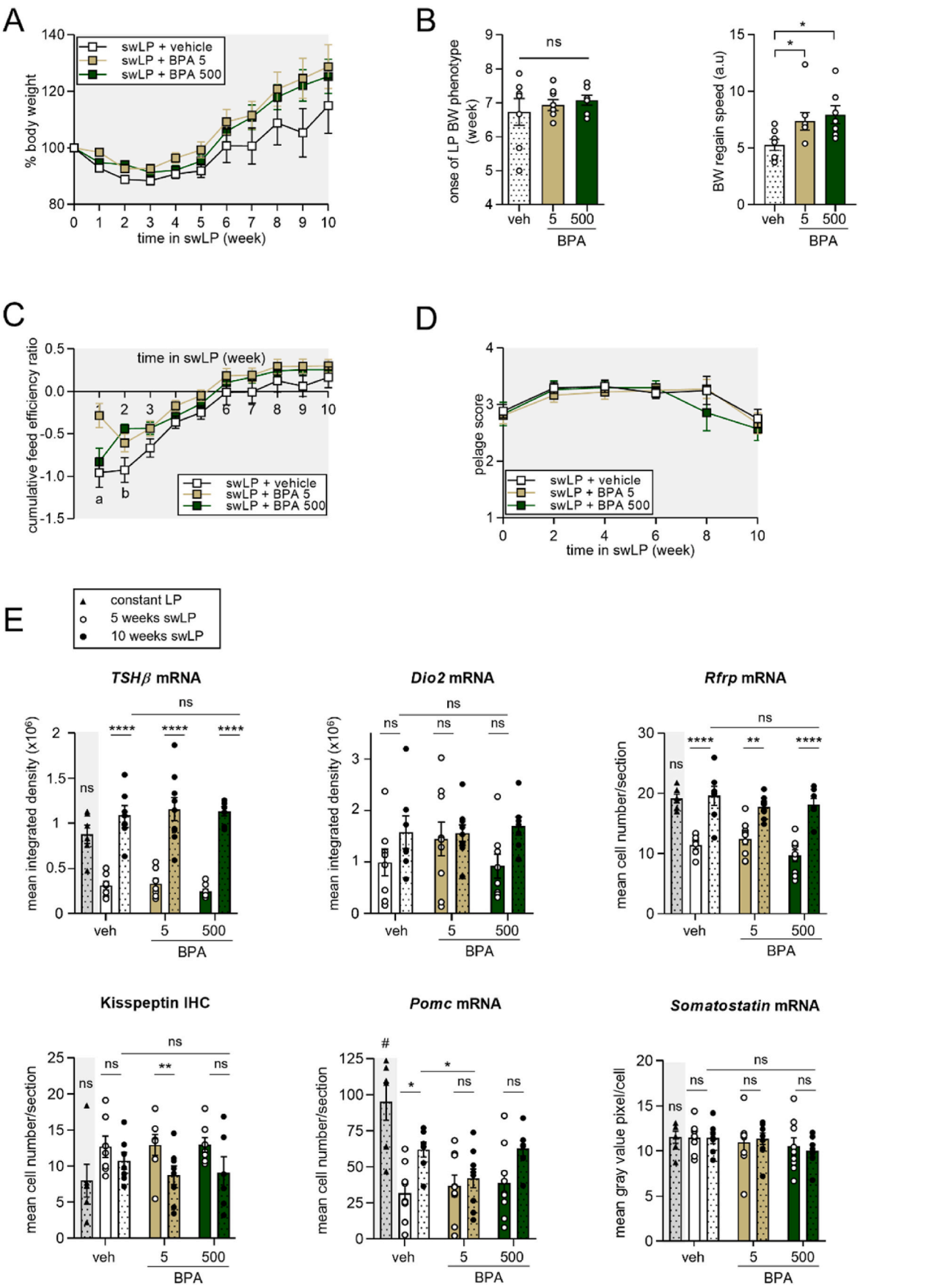

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Fig. 5. Effects of BPA exposure on body weight, food intake, pelage whitening, and various photoperiodic genes/proteins in female Djungarian hamsters transferred from short to long photoperiod. Female Djungarian hamsters adapted to short photoperiod (SP) were switched back in long photoperiod (swLP) and either fed with pellet food containing 0.02% ethanol (swLP + vehicle; n = 8–17) or containing 5 (swLP + BPA 5; n = 9–18) or 500 μg (swLP + BPA 500; n = 7–16) BPA/kg/day. **(A)** Body weight (BW) expressed in percentage of the SP value (before the swLP transfer). **(B)** Time in weeks to reach the LP body weight phenotype (point of inflection calculated from a non-linear regression model fitted to individual body weight raw data) and speed of LP-induced body weight gain (slope of the non-linear regression). **(C)** Cumulative feed efficiency ratio over 10 weeks of swLP. **(D)** Pelage score (from 1 being a LP-adapted grey color to 5 being a SP-adapted white color) over 10 weeks of swLP. **(E)** Expression of *pars tuberalis TSHβ*, and hypothalamic *Dio2*, *Rfrp*, *Pomc*, and *somatostatin* gene and kisspeptin protein in constant LP (grey bar) or after 5 (empty bar) and 10 (dotted bar) weeks of swLP. Values are given as mean ± SEM (n = 7 to 18 according to experimental groups). Fig. 5C: Statistical significance: for repeated values over time, *a*, $p < 0.05$ between swLP + BPA 5 vs swLP + vehicle, *b*, $p < 0.05$ between swLP + BPA 50 vs swLP + vehicle; Fig. 5A & E: for multiple comparisons between groups at the 10^{th} week, *, $p < 0.05$ vs swLP + vehicle; for multiple comparisons of the dynamic changes of mRNA/protein expression between the 5^{th} and the 10^{th} week for each dose, *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$; to ascertain the photoperiodic effects on gene/protein expression, when possible comparisons were made between the LP + vehicle and swLP + vehicle groups (# when $p < 0.05$). ns = no significance; veh = vehicle.

Table 2
Effects of BPA exposure on reproductive and metabolic parameters in female and male Djungarian hamsters transferred from short to long photoperiod. Hamsters were orally exposed (through food) to 0.02% ethanol (swLP + vehicle; n = 8–11), or to 5 (swLP + BPA 5; n = 9–11) or 500 (swLP + BPA 500; n = 7–12) μg of BPA/kg/day after a 10-week transfer from a short to a long photoperiod (swLP), or when kept under long photoperiod conditions (LP + vehicle; n = 8–10). Values are indicated as mean ± SEM. Statistical significance: #, $p < 0.05$ between LP + vehicle vs swLP + vehicle (10^{th} week), *a*, $p < 0.05$ between swLP + BPA 5 vs swLP + vehicle.

photoperiod/dose	females							males			
	LP + vehicle	swLP + vehicle		swLP + BPA 5		swLP + BPA 500		LP + vehicle	swLP + vehicle	swLP + BPA 5	swLP + BPA 500
weeks	- (n = 8)	5^{th} (n = 9)	10^{th} (n = 8)	5^{th} (n = 9)	10^{th} (n = 9)	5^{th} (n = 9)	10^{th} (n = 7)	- (n = 10)	10^{th} (n = 11)	10^{th} (n = 11)	10^{th} (n = 12)
relative reproductive organ weight (mg/g of body weight)	2.5 ± 0.2#	1.6 ± 0.1	4.6 ± 0.5	2.1 ± 0.2a	3.6 ± 0.5	1.3 ± 0.1	3.7 ± 0.7	20.3 ± 1.8	18.9 ± 1.1	20.9 ± 1.8	19.4 ± 1.2
estradiol (pg/ml of plasma)	283 ± 63	240 ± 42	367 ± 106	171 ± 42	388 ± 90	159 ± 35	375 ± 60				
testosterone (pg/ml of plasma)								35.8 ± 15.7	11.8 ± 3.5	33.4 ± 13.9	13.8 ± 6.5
glucose (mg/dl of blood)	192 ± 12	174 ± 48	178 ± 13	179 ± 15	186 ± 20	166 ± 13	186 ± 20	157 ± 6	156 ± 15	190 ± 19	146 ± 9

associated with this BPA-induced metabolic alteration, but the glucose/insulin ratio was increased in male hamsters exposed to 5 μg/kg/day BPA as compared to control animals. This suggests an increase in insulin sensitivity and a reinforcement of the central anorexigenic effect of insulin (Kauffman and Castracane, 2003), as insulin can inhibit orexigenic NPY neurons (Lee and Herzog, 2021).

In order to better understand the metabolic properties of BPA in Djungarian hamsters, further investigations on the central regulation of metabolic neurons, notably their sensibility to leptin (Mackay et al., 2017), and on peripheral organs (liver, pancreas, adipose tissue, reported to be sensitive to BPA (Marmugi et al., 2012; Soriano et al., 2012; García-Arevalo et al., 2014)) are required.

4.1.2. BPA modulates the kinetics of SP-induced Fur whitening in both female and male hamsters

Melatonin controls photoperiodic changes in fur color and density in seasonal rodents through the regulation of prolactin secretion (Hoffmann, 1973; Duncan et al., 1985; Rose et al., 1985). Exposure to 5 or 500 μg BPA/kg/day during the transfer from LP to SP resulted in a delay in females and an advance in males of fur whitening. BPA has been shown to promote prolactin secretion in pituitary tumor cell lines (Wozniak et al., 2005; Chun and Gorski, 2000), but it may also act as a blocker of prolactin secretagogues in bullfrog pituitaries (Kaneko et al., 2008). Although technically challenging, the longitudinal analysis of the photoperiodic change in prolactin secretion could help to determine whether this sex-dependent effect of BPA on pelage changes occurs at the level of prolactin synthesis or on downstream mechanisms. One study in Agouti mice reported that BPA exposure induced a change in coat color mediated by a reduction in methylation of the Agouti gene in germ cells (Dolinoy et al., 2007). During the 10-week transfer from SP to LP, the fur color did not change significantly, independently of BPA exposure, possibly because the prolactin levels were still low for all the experimental groups, as the spring color change occurs only in hamsters in which prolactin levels are restored (Duncan and Goldman, 1984).

4.1.3. A low dose of BPA accelerates the LP-induced restoration of reproduction in female hamsters

A non-invasive and not stressful longitudinal monitoring of reproductive activity is challenging in the Djungarian hamster. Thus, the assessment of BPA's effect on the kinetics of photoperiodic changes in reproductive activity could only be conducted in female hamsters transferred from SP to LP and sampled at an intermediate stage (5^{th} week of swLP). Exposure to 5 μg BPA/kg/day, but not 500 μg BPA/kg/day, accelerated uterine and ovarian weight gain compared to vehicle-treated female hamsters. Previous studies in birds have shown that other EDCs, specifically isoflavones, could also alter the dynamics of reproductive responses to changes in photoperiod. Notably, a diet enriched in isoflavones delayed the growth of the cloacal protuberance of oscines in response to an LP transfer (Corbitt et al., 2007) and reduced the testicular development of Japanese quails transferred from an inhibitory SP to a stimulatory LP (Wilhelms et al., 2006). To confirm that BPA exposure can alter the kinetics of the photoperiodic regulation of reproductive activity, complementary studies incorporating more intermediate sampling times should be conducted in male and female hamsters. These additional sampling points could help elucidate the effects of BPA on the hormonal milieu, which undergoes significant changes during photoperiodic adaptations. Indeed, male and female Djungarian hamsters transferred to SP experience the inhibitory effects of prolonged melatonin secretion on hypothalamic thyroid hormone activity, which subsequently reduces the hypothalamic synthesis of gonadotropin-releasing hormone (GnRH) and, in turn, the pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Buchanan and Yellon, 1991; Yellon and Goldman, 1987). These endocrine alterations ultimately lead to gonadal weight

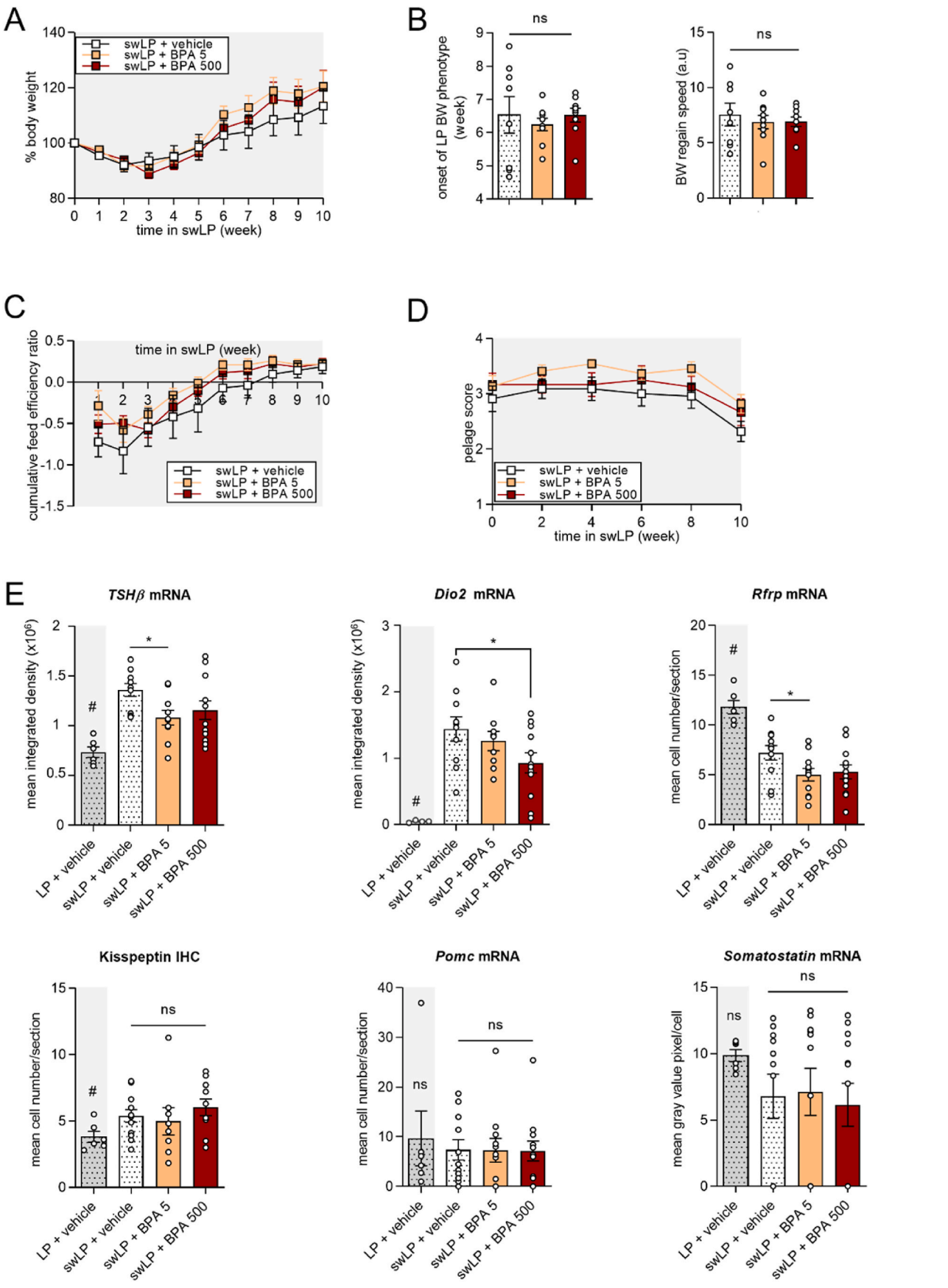

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Fig. 6. Effects of BPA exposure on body weight, food intake, pelage whitening, and various photoperiodic genes/proteins in male Djungarian hamsters transferred from short to long photoperiod. Male Djungarian hamsters adapted to short photoperiod (SP) were switched back in long photoperiod (swLP) and either fed with pellet food containing 0.02% ethanol (swLP + vehicle; n = 11) or containing 5 (swLP + BPA 5; n = 11) or 500 (swLP + BPA 500; n = 12) μg BPA/kg/day. **(A)** Body weight (BW) expressed in percentage of the value before the swLP transfer. **(B)** Time in weeks to reach the LP body weight phenotype (point of inflection calculated from a non-linear regression model fitted to individual body weight raw data); and speed of LP-induced body weight gain (slope of the non-linear regression). **(C)** Cumulative feed efficiency ratio over 10 weeks of swLP. **(D)** Pelage score (from 1 being a LP-adapted grey color to 5 being a SP-adapted white color) over 10 weeks of swLP. **(E)** Expression of *pars tuberalis TSHβ*, and hypothalamic *Dio2*, *Rfrp*, *Pomc*, and *somatostatin* gene and kisspeptin after 10 weeks of swLP, or constant LP (grey bar). Values are given as mean ± SEM (n = 11 to 12 according to experimental groups). Statistical significance: for multiple comparisons between groups (B, E), *, $p < 0.05$ vs swLP + vehicle; to ascertain the photoperiodic effects on gene/protein expression, when possible, comparisons were made between the LP + vehicle and swLP + vehicle groups (# when $p < 0.05$). *ns* = no significance; veh = vehicle.

regression, which correlates well with gonadal inactivity (Schlatt et al., 1995; Sinha Hikim et al., 1988; Moffatt-Blue et al., 2006), and results in a dramatic decrease in circulating sex steroid concentrations (Moffatt-Blue et al., 2006; Yellon and Goldman, 1987; Furuta et al., 1994). Given the multiple modes of action of BPA on hormone synthesis, transport, distribution, clearance, and hormone receptors (La Merrill et al., 2020), the observed effects of BPA on physiological photoperiodic adaptations may be explained by its interactions with the changing hormonal environment. Although measurements of plasma estradiol and testosterone at the end of the photoperiodic transfers indicated changes in the hormonal milieu (with a strong decrease in SP and a strong increase in LP), no significant differences were observed between the experimental groups. Longitudinal measurements of sex steroid hormones, but also pineal melatonin, hypothalamic T3/T4, gonadotropins, and the expression of their receptors in relevant tissue (such as *pars tuberalis*, hypothalamic nuclei, and gonads) should enhance our understanding of the mechanisms of action of BPA in the dynamic of seasonal adaptation. Conversely, changes in the hormonal milieu and hormone receptor expression can also influence the effects of BPA, as some of BPA's modes of action depend on the availability of circulating and tissue hormones, as well as the presence of hormone receptors on tissues (La Merrill et al., 2020). Therefore, it is not surprising to observe different impacts of BPA on hamsters of the same sex exposed to the same dose, depending on their photoperiodic conditions. For instance, females exposed to 5 μg BPA/kg/day during the transferred to SP exhibited a normal transition to a winter-like phenotype, whereas those transferred back to LP showed a faster acquisition of the reproductive summer-like phenotype.

Recent studies in Djungarian hamsters have reported that the photoperiodic control of reproductive activity depends on ARC kisspeptin regulation by melatonin and sex steroids and that kisspeptin can restore SP-inhibited gonadal activity (Rasri-Klosen et al., 2017; Cázarez-Márquez et al., 2019). Interestingly, the early reactivation of the reproductive axis in females exposed to BPA during the LP transfer was associated with an advance in the reduction of ARC kisspeptin compared to control females. Previous studies have also reported that BPA can reduce kisspeptin ARC expression in rats (Patisaul et al., 2009) and mice (Ruiz-Pino et al., 2019) and suppress kisspeptin liberation in the median eminence of rhesus monkeys (Kurian et al., 2015). As ARC ERα expression can also be altered by BPA exposure (Cao et al., 2012; Monje et al., 2010) one hypothesis could be that BPA may induce an early sensitization of the ARC kisspeptin neurons to the negative feedback of circulating estrogens, which are already high at the 5th week of swLP.

4.1.4. Potential ecological impact of BPA exposure in Djungarian hamsters

It is important to note that the experimental design may not fully replicate the environmental changes encountered by seasonal mammals in nature, as there is limited data available on the actual BPA exposure levels in terrestrial wildlife. However, the doses used in the study spanned those based on regulatory guidelines, ranging from the tolerable daily intake of 4 μg/kg/day to 10 times lower than the NOAEL of 5 mg/kg/day.

Although the photoperiodic changes were abrupt, lacking the natural gradual decrease or increase in day length, our study reported clear BPA effects on the dynamics of photoperiodic adaptation in male and female Djungarian hamsters. This raises important concerns about potential ecological consequences of BPA exposure in seasonal species. The acceleration of physiological adaptations to the winter photoperiod, as observed in BPA-exposed male hamsters, or to the summer photoperiod, as observed in BPA-exposed female hamsters, can be interpreted as an adaptive advantage, enabling the individuals to anticipate their physiology more quickly for the upcoming season. Nevertheless, animals may temporarily find themselves in a discordant physiological state with the seasonal photoperiodic change in their environment.

Our study also highlighted a striking sexual dimorphism in the BPA-induced change in the kinetics of photoperiodic integration, notably from LP to SP conditions where females showed a delay while males showed an advance in their metabolic adaptation. The consequences of such mismatches, even if temporary, between the physiological state of an individual, the resources of its environment, and the physiological state of its conspecifics may lead to impaired species adaptation to seasons on a population level.

5. Conclusion

This study is the first to reveal significant effects of BPA exposure on the photoperiodic adaptation of a mammalian species, namely the Djungarian hamster. The findings demonstrate that BPA exposure disrupts the kinetics of metabolic and reproductive responses to photoperiodic changes in a markedly sex-dependent manner. These effects were dependent on the doses, which, although chosen according to regulatory guidelines, might not accurately reflect the actual environmental exposure levels of wildlife. Seasonal adaptation driven by photoperiodic changes relies on a complex photoneuroendocrine pathway involving the melatoninergic system, hypothalamic thyroid hormones and neuropeptides, and sex steroids, all of which are key targets for BPA and other EDCs. While the effects of BPA on seasonal physiology were associated with several neuroendocrine changes, the use of non-invasive approaches to track physiological responses over time limited a detailed mechanistic understanding of these effects. Future research should assess the long-term consequences of EDCs exposure on seasonal adaptation, particularly when exposure occurs during critical windows of vulnerability in early life. Finally, it is important to investigate the effects of EDCs exposure on the integration of other ecological seasonal factors –such as ambient temperature, food resources, and humidity– which can also influence seasonal physiology.

CRediT authorship contribution statement

Marie-Azélie Moralia: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Béatrice Bothorel:** Supervision, Methodology, Investigation. **Virginie Andry:** Methodology. **Yannick Goumon:** Methodology. **Valérie Simonneaux:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143955.

Data availability

Data will be made available on request.

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