The effect of prenatal environment on brain metabolic function and perception in a lizard

## Abstract

Cognitive processes such as the ability to perceive prey, are crucial for survival and reproduction. Early-life conditions like stress-related hormones or thermal environments, can shape cognitive abilities by influencing brain structure and function. Mitochondrial physiology is thought to be a central mechanism underlying these effects given that it is crucial for the energy production needed for brain function. Here, we investigated the combined influence of prenatal corticosterone (CORT)–a key stress hormone in reptiles–and incubation temperature on brain mitochondrial function and impacts on prey detection in the delicate skink (*Lampropholis delicata*). We manipulated egg CORT levels and incubation temperature, then assessed the ability of lizards to detect chemical and visual prey stimuli. Using flow cytometry, we measured metabolic function, reactive oxygen species (ROS) production, and oxidative stress in the olfactory bulbs and optic tecta-two brain regions involved in sensory processing. While metabolic function remained robust to early life conditions, CORT and temperature interacted to influence oxidative stress. Additionally, CORT-treated lizards responded faster when presented with chemical cues but not visual stimuli. Our findings suggest that prenatal conditions can have lasting effects on brain physiology and cognition, highlighting a surprising decoupling between mitochondrial function and perception in lizards.

## Introduction

Cognition encompasses the ways in which animals acquire, process, and store information, enabling perception, learning, memory, and decision-making [[1](#ref-shettleworth)]. It is essential for survival and reproduction, allowing individuals to adapt to changing environments [[2](#ref-dukas_evolutionary_2004)]. However, cognitive abilities can vary considerably between individuals, with differences arising from genetic factors, environmental conditions, or a combination [[2](#ref-dukas_evolutionary_2004),[3](#ref-sakata_neural_2000)]. The prenatal environment, in particular, plays a critical role in shaping brain development and cognition across a wide range of species [[3](#ref-sakata_neural_2000)–[6](#ref-amiel_effects_2017)]. However, the impact of early-life environments has primarily been studied in the context of learning and memory [[4](#ref-zhu_prenatal_2004),[5](#ref-crino_corticosterone_2014-learn),[7](#ref-bebus_associative_2016),[8](#ref-abayarathna_effects_2020)], neglecting how other important cognitive domains might be affected [but see [9](#ref-burger_antipredator_1998),[10](#ref-vila_pouca_quantity_2019)]. In this vein, perception is an often overlooked, but fundamental cognitive process, involved in behaviours critical to survival and reproductive success such as locating food, avoiding predators, or interacting with conspecifics [[1](#ref-shettleworth),[9](#ref-burger_antipredator_1998),[11](#ref-hairston1982fish)–[14](#ref-recio2023conspecific)]. Understanding how prenatal conditions affect the ability to detect different stimuli can be fundamental to understanding the broader consequences of early-life environments on fitness.

Prenatal environments influence cognitive abilities because the brain is particularly sensitive during early stages of development [[4](#ref-zhu_prenatal_2004)]. Early-life conditions can shape cognition by altering gene expression [[15](#ref-zhou2020effects)], neurotransmitter production and neuroendocrine pathways [[16](#ref-amani2021perinatal)], or brain structure and function [[6](#ref-amiel_effects_2017)]. Additionally, developmental environments can have long-lasting effects on energy production and oxidative stress in the brain, which may be a key mechanism influencing cognitive function [[17](#ref-siegel1994basic)–[20](#ref-picard_energetic_2018)]. First, given the high energetic demands of cognitive processes [[21](#ref-mcnay_decreases_2000)–[23](#ref-alexandrov_neuronal_2022)], cognitive performance is intrinsically linked to efficient mitochondrial respiration. Likewise, high cognitive abilities are typically associated with increased neuron density and functionality [[6](#ref-amiel_effects_2017),[24](#ref-lefebvre_taxonomic_2011)], both of which can be impaired by excessive reactive oxygen species (ROS) production and oxidative stress [[4](#ref-zhu_prenatal_2004),[18](#ref-du_dynamic_2009),[25](#ref-finkel_oxidants_2000)–[27](#ref-hoffmann_mitochondrion_2018)]. For instance, Hara et al. (2014) found that performance in visuospatial working-memory tasks is negatively correlated to the number of round-shaped mitochondria-associated with higher oxidative stress-per presynaptic terminal. Thus, understanding how early-life conditions affect mitochondrial physiology may provide insights into the mechanisms underlying cognitive performance.

Mitochondria are highly responsive to environmental conditions during early development, with potential short-term and long-lasting consequences [reviewed in [28](#ref-gyllenhammer2020developmental)]. For example, environmental conditions or hormone exposure can cause immediate disruptions to metabolic function, triggering cascading effects on oxidative stress that may persist later in life [reviewed in [28](#ref-gyllenhammer2020developmental)]. Among the range of environmental factors that can shape mitochondrial physiology, some exert particularly strong and consistent effects. Stress-related hormones and temperature are particularly relevant for mitochondrial function due to their strong effects on metabolic processes [[29](#ref-sapolsky_how_2000)–[31](#ref-crino2024eggs)]. Vertebrates respond to stressful situations through the activation of physiological processes including the production of glucocorticoids (GCs), which are also important metabolic regulators [[29](#ref-sapolsky_how_2000)]. In ectothermic vertebrates, temperature plays a similar role in regulating metabolic activity [[30](#ref-stier2022experimental),[31](#ref-crino2024eggs)]. In fact, heightened GC levels or changes in the thermal environment during early development have profound and sustained effects on mitochondrial respiration efficiency or oxidative stress [[4](#ref-zhu_prenatal_2004),[30](#ref-stier2022experimental)–[35](#ref-treidel2016temperature)]. However, how the early environment influences mitochondrial physiology and its associated cognitive consequences remains largely unknown outside of mammals [see [36](#ref-chaudhari2022early) for a review]. In addition, whether and how these interactions affect perceptual abilities, such as prey detection, is still largely unexplored.

Here, we investigated the combined effects of prenatal temperature and corticosterone (CORT) - the main GC in reptiles - on mitochondrial physiology and prey detection in a lizard (*Lampropholis delicata*). We manipulated egg CORT levels and incubation in a fully factorial design. We then assessed hatchling prey detection ability and quantified metabolic function in brain regions related to processing chemical and visual cues. We hypothesized that prenatal CORT exposure and the incubation thermal environment would influence metabolic function and oxidative stress in the brain [[4](#ref-zhu_prenatal_2004),[30](#ref-stier2022experimental)–[35](#ref-treidel2016temperature)], with significant repercussions on cognition [[4](#ref-zhu_prenatal_2004),[37](#ref-hara_presynaptic_2014)]. We predicted that metabolic deficiencies or increases in oxidative stress and damage would result in lower cognitive abilities (i.e. longer time to detect prey) [[20](#ref-picard_energetic_2018),[23](#ref-alexandrov_neuronal_2022)]. Furthermore, we predicted prenatal conditions to have region-dependent effects on mitochondrial function [[38](#ref-coomber_independent_1997)] that would lead to stimulus-specific differences in prey detection.

## Methods

#### Animal husbandry

*Breeding colony* – The lizards tested came from a breeding colony established in the laboratory in 2019. The colony consisted of 270 adults of *L. delicata* housed in plastic containers (41.5 L x 30.5 W x 21 H cm) with six lizards (two males and four females) per enclosure. Enclosures were provided with non-stick matting, shelter, and several small water dishes. Water was given daily, and lizards were fed approx. 40 mid-size crickets (*Acheta domestica*) per enclosure three days a week. Crickets were dusted with calcium weekly and multivitamin and calcium biweekly. Room temperatures were set to 22-24 ºC, but enclosures were also provided with a heat chord and a heat lamp following a 12 h light:12 h dark cycle keeping warm side of enclosures is usually at 34 ºC.

*Egg collection and incubation* – Between mid-October 2022 and the end of February 2023, we provide females with a place to lay the eggs by placing a small box (12.5 L x 8.3 W x 5 H cm) with moist vermiculite in one side of the communal enclosures. These boxes were checked three days a week for eggs. After collection, eggs were treated with either CORT or a vehicle control (see CORT and temperature manipulation below) and placed in individual cups (80 mL) with moist vermiculite (12 parts water to 4 parts vermiculite). Cups were covered with cling wrap to retain moisture and left at one of two different incubation temperatures (see CORT and temperature manipulation below) until hatching.

*Hatchlings* – Incubators were checked three times a week for hatchlings. Hatched lizards were placed in individual enclosures (18.7L x 13.2W x 6.3H cm) provided with nonstick matting and a small water dish until the beginning of the experiment. During this period, lizards were given water daily and received 3-6 small *A. domestica* crickets three times a week. All care otherwise follows similar protocols to adults (see above).

#### CORT and Temperature manipulation

To test the interactive effects of CORT and incubation temperature, we manipulated CORT concentrations in eggs and incubated them under one of two temperature regimes (see [Fig. 1](#fig-Methods) A). We used a partial split clutch design where eggs from a given clutch were distributed equally across the four treatments when clutch sizes were larger than four and randomly across treatments when less than four. Eggs were topically supplied with either: a) 5 µL of crystalline corticosterone (Sigma, Cat. No. C2505) dissolved in 100% ethanol at a final 10 pg CORT/mL concentration (CORT treatment), or b) an equal volume of 100% Ethanol (Control treatment). We selected doses based on previous studies where CORT treatment increased mean yolk CORT levels by approximately 2 standard deviations above the mean natural concentration [[31](#ref-crino2024eggs)]. Eggs were then incubated at either cold (23 ± 3 ºC) or hot (28 ± 3 ºC) (see [Fig. 1](#fig-Methods) A). These temperatures are within the natural limits in *L. delicata* [[39](#ref-cheetham2011embryonic)].

#### Prey discrimination tests

Two weeks before we started the tests (see below), lizards were moved to the experimental arena (see Fig. 1) for acclimatization. The arenas were individual medium size (41 L x 29.7 W x 22 H cm) plastic containers with a shelter (9 L x 6 W x 1.5 H cm) on one of the extremes and a water dish in the middle of the arena. Arenas were placed in two rooms on six racks, each with its own CCTV system (device model DVR-HP210475) that allowed us to record lizard behaviour during the experiment (see details below). The number of lizards per treatment in each rack was counterbalanced to control for any effect of the room or the position of the lizard on the rack. During acclimatization, lizards were fed with only one cricket per day dusted with calcium and multivitamin, and water was supplied *ad libitum*. We provided a temperature gradient by means of a heat cord and heat lamps in a 12 h light: 12 h dark cycle. The room temperature was set to between 22-24 Celsius. After the tests, animals were euthanized and metabolic function was analyzed in various brain regions (see Brain mitochondrial activity protocol below).

The experiment involved presenting lizards with chemical and visual stimuli from different familiar and unfamiliar prey, then recording and analyzing their behaviour towards each stimulus ([Fig. 1](#fig-Methods) C). We used crickets (*A. domestica*) as the familiar prey and mealworm larvae (*Tenebrio molitor*) as the unfamiliar prey. We expected to see differences between known and unknown prey because previous experience may influence stimuli perception through habituation or sensitisation [[13](#ref-desfilis2003stimulus),[40](#ref-burger_effects_1990),[41](#ref-burger_effects_1991)], and we included familiarity with the prey as a factor in our analyses (see below).

Each stimulus was presented inside a transparent plastic vessel containing a white, two-chambered device (see [Fig. 1](#fig-Methods) B) made of polylactic acid (PLA). In chemical trials, the prey was placed in the closed chamber at the back of the device, making it invisible to the lizard, while in visual trials, the prey was placed in the front chamber. Holes in both the device and the front sides of the transparent vessel (see [Fig. 1](#fig-Methods) B, C) allowed chemical cues to be released; however, these holes were sealed with silicone in the visual trials. To increase the availability of chemical cues, we glued a piece of filter paper (left for at least 8 hours in one of the prey’s enclosures: *A. domestica* or *T. molitor*) to the device during chemical trials. In visual trials, the filter paper was placed in an empty box for the same duration under identical conditions. In both chemical and visual trials, the prey remained inside the vessel to control for potential acoustic cues. The order of stimulus presentation was counterbalanced across treatments.

Each trial began by placing the experimental device at the side of the arena opposite to the shelter (see arena in [Fig. 1](#fig-Methods) A), and then removing the shelter. The water cup had already been removed. We recorded the lizard’s behaviour for approximately one hour. We assessed lizards’ ability to detect each stimulus (‘Detection latency’) by recording the time from when the lizard resumed normal activity (i.e., walking for at least 5 consecutive seconds; T0 in [Fig. 1](#fig-Methods) D) until the first interaction with the stimulus (TD in [Fig. 1](#fig-Methods) D). Lizards were considered to have interacted with the object when the lizard touched the front of the vessel or the filter paper with its snout for more than five consecutive seconds.

To control for potential differences in hunger levels, all lizards were fasted for two days before the experiment, a period considered harmless for this species [[42](#ref-young2022physiological)]. After each trial, the lizards were also given a cricket to assess their motivation to forage. The cricket was left in the enclosure for one hour, and we recorded whether the lizard ate it (recorded as 1) or not (recorded as 0). In 23 videos, the camera stopped recording before the end of the motivation test (Tf in [Fig. 1](#fig-Methods) D), so motivation was recorded as NA. We used their performance in the motivation test as a covariate in the analyses (see below).

All trials were conducted between 1100 and 1300 h, when the lizards were most active. To control for potential effects of neophobia, we simulated test conditions for two days prior to the experiment by removing the shelter and water cup, and exposing the subjects to the vessel without a stimulus. This simulation lasted for 1 hour at the same time of day as the tests, but no behavior was recorded.

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| Fig 1— Scheme of our experimental design. In panel A, we show the different stages of our experiment and the main manipulations. In panel B, we show the experimental device used to present the stimuli in the behavioural tests. Here, F indicates the front of the device, and Bk the back. In panel C, we show the experimental setup for the prey discrimination tests. In panel D, we show the relevant times from our behavioural tests. |

#### Brain metabolic function

*Brain dissection and homogenization*: Two months after the completion of the tests, we euthanized lizards using an injectable anaesthetic followed by decapitation. We injected intraperitoneally 10 mg/kg of a 10 mg/mL alfaxan solution and then, after several minutes, we evaluated the lizard’s righting response and pinching reflex in one of the front limbs. Lizards without responses were decapitated with surgical scissors. This protocol was approved by the Animal Ethics Committee of the Australian National University (Protocol number: A2022/33). After decapitation, the head was opened and the brain was dissected. We extracted two main regions of the brain, the olfactory bulbs and the optic tecta, as they are associated with chemical and visual perception in lizards [[43](#ref-wyneken2007reptilian)]. Both regions were transferred immediately to 1.5mL centrifuge tubes containing 100µL of 1X phosphate buffered saline (PBS).

Tissue suspented in PBS was then homogenized. For the olfactory bulbs, the tissue was mechanically homogenized by placing the tissue in the well of a 100 µm mesh filter (pluriStrainer) affixed atop a 1.5 mL centrifuge tube, then mashed with the rubber end of an insulin syringe stopper. The resulting olfactory bulb homogenate was then rinsed through the filter with 1 mL of cold 1XPBS. Optic tecta were mechanically homogenized the same way as olfactory bulbs, but were first enzymatically digested by incubating the tissue in 100µL of 125 U/mL collagenase (type II).

Homogenates were split among two aliquots: one was used fresh to measure mitochondrial density, membrane potential - a metric of metabolic capacity [[44](#ref-martinez2016tca)] - and ROS, while the other one was cryopreserved for later measurements of DNA damage and lipid peroxidation. Cryopreservation was made by suspending the homogenates in 1 mL solution of 1% Neutral-Buffered Formalin, 1X Tris-EDTA, and 10% DMSO, then stored at -20 °C until oxidative damage assays.

*Metabolism and ROS*: Fresh homogenate suspensions were stained with 5 µL of a fluorescent probe mix containing equal parts 5 µM MitoTracker Deep Red FM, 2.5 µM MitoTracker Orange CMTMRos, and 50 µM MitoSOX Red. We used these fluorescent probes as indicators of mitochondrial density, metabolic capacity, and superoxide (ROS) production, respectively. We also added 10 µg/mL Hoechst 33342 Nuclear Viability Dye to each sample, which we used to distinguish live, viable, intact cells from cellular debris. Within two hours after dissection, these samples were analyzed by flow cytometry (Becton Dickson LSRFortessa X-20).

*Oxidative Damage*: Assays of oxidative damage from cryopreserved samples were performed 61 weeks after the initial processing and analysis of fresh samples. On the day of oxidative damage assays, we rapidly thawed frozen samples, removed the cryopreservation solution (see above), and resuspended samples in 1 mL warm 1X Tris-EDTA. Afterward, the Tris-EDTA was removed, and samples were resuspended in 200 µL of warm 1X PBS containing 10 µg/mL Hoechst 33342 Nuclear Viability Dye (for cell viability) and 100 µM BODIPY 665/676 Lipid Peroxidation Sensor (to measure lipid peroxidation). Following staining, we permeabilized the cell membranes incubating the samples in 200 µL warm 1X PBS containing 20 µM digitonin. Following permeabilization, we stained the samples with 20 µL of 70 µM 8-OHdG Polyclonal Antibody to measure oxidative damage on DNA. We left the homogenate overnight (~12 hours) at 4 ºC. The following day we counterstained the cells with 20 µL of 100 µg/mL H+G Goat Anti-Rabbit Conjugate Antibody with Alexa-Fluor 488 and analyze the samples in the flow cytometer.

*Flow Cytometry*: Flow cytometry assays were performed using a Becton Dickson LSRFortessa X-20 flow cytometer with the default wavelength filters on detectors. The detectors and voltage settings used in data acquisition for each were determined during pilot trials and kept consistent throughout the experiment. Data was imported into FlowJo (v. 10.1) for processing. We obtained the mean fluorescent intensity for mitochondrial density, metabolic capacity, ROS production, DNA damage, and lipid peroxidation. For further details on the homogenization, staining, or flow cytometry assays, see Methods: flow cytometry in Supplementary Material. We validated that our homogenates contained neurons in a pilot study using dyes specifically targeting neuronal nuclei (See *Brain validation in Supplementary Material*). This pilot study also ensured that our gating strategy identified these neurons using Flow cytometry.

We recorded five physiological variables from our flow cytometry assay: mitochondrial density, metabolic capacity, ROS production, DNA damage, and lipid peroxidation. These variables reflect average per-cell values for each sample, as fluorescence intensity was normalized by the number of gated events (cells) recorded. However, to assess how early environmental conditions may influence mitochondrial function at the organelle level, we created two additional response variables by dividing metabolic capacity and ROS production by mitochondrial density. This procedure assumes that mitochondrial density serves as a proxy for the number of mitochondria per cell, and the derived variables provide an estimate of the average activity per mitochondrion, therefore allowing us to assess mitochondrial-specific responses to early-life conditions.

#### Statistical analyses

We performed the analyses for each brain region/stimulus and each variable (mitochondrial density, metabolic capacity, metabolic capacity/mitochondrial density, ROS, ROS/mitochondrial density, DNA damage, lipid peroxidation, and detection latency) separately. We first fit a set of preliminary models for all the interest variables where we included the hormone (CORT versus Control), temperature (Cold versus Hot), and their interaction. These models also included the sex and age of the lizards at the time where the trials started (for detection latency) or when the lizards were euthanized (for all mitochondrial-related variables). For detection latency, we also included previous experience with the prey (familiar versus unknown), their performance on the motivation test [if they ate the cricket (1), or not (0)], and the interaction between CORT and motivation as fixed effects. We included the interaction between CORT and motivation because CORT can impact appetite [[45](#ref-conde2018stress)]. All models included clutch identity as a random factor to account for clutch effects. For models of detection latency we also included a random effect of lizard identity because of our repeated measures design. The structure and results of these models are provided on Tables S1-S22 in *Supplementary Material*. After the preliminary models, we simplified models by excluding some of the factors that were not significant. However, we always included the main interest factors: CORT, temperature and their interaction. Random factors remain the same for the final models. All the response variables were mean centered and standardized by dividing by two times the standard deviation [[46](#ref-gelman2008scaling)]. Before standardization, mitochondrial density, DNA damage, lipid peroxidation, and detection latency were log-transformed.

All models were fit using using the package *brm* which insterfaces with Stan [[47](#ref-stan)] in R (version 4.4. 0) [[48](#ref-R)]. The error structure was modelled assuming a Gaussian distribution for all variables. We ran four parallel MCMC chains of 8000 iterations for each model, with a warmup period of 2000 iterations. To test for differences between treatments, we made contrasts between treatments using the posterior distribution of relevant parameters. Significance was assessed by using the posterior distributions of parameter estimates and contrasts to test whether were they different from zero [[49](#ref-endo2019introduction)]. We considered an effect statistically significant if pMCMC < 0.05.

To further explore the relationships between mitochondrial physiology and detection latency, we used a Structural Equation Modelling (SEM) approach. We fit a multivariate brm from stan [[47](#ref-stan)] in R (version 4.4.0) [[48](#ref-R)] for each brain region/stimulus separately. We included in the model all the variables of interest and their interactions structured following a specific set of hypotheses (see [Fig. 5](#fig-sem_results_OB) and [Fig. 6](#fig-sem_results_OT)). Because experience with prey did not affect lizards’ behaviour (see Tables S21, S22), to reduce the complexity of the models, we averaged lizards detection latency across both types of prey and excluded lizard identity from the random factors. Clutch identity was included as a random factor for all variables. For our SEM model, the error structure was modelled assuming a Gaussian distribution and we estimated residual correlations among all variables.

We obtained direct, indirect, and total effects from posterior distributions of parameters estimated in the multivariate models. Direct effects represent the posterior estimates of a predictor’s effect on the response variable, while indirect effects were computed as the product of the direct effects along the path [[50](#ref-kline2005principles)]. Total effects are the sum of direct and indirect effects.

## Results

Our final sample size for mitochondrial assays was 80 lizards from a total of 50 clutches (n = 20 per treatment). Each lizard was subjected to 4 tests for a total of n = 320 behavioural observations (n = 2 with missing data).

#### Do prenatal CORT and temperature affect mitochondrial function and oxidative stress in the brain?

Models explained between 37.8 to 50.4 % of the variation in mitochondrial function and 39.9 to 59 % for oxidative stress (see Table S1).

*Olfactory bulbs*: Age significantly increased DNA damage and lipid peroxidation (Table S3). However, we did not find any significant effects of CORT, temperature on mitochondrial function nor oxidative stress in the olfactory bulbs (see [Fig. 2](#fig-results_energy), [Fig. 3](#fig-results_oxidative), and Table S2). The posterior distribution provided little support for an overall CORT-temperature interaction on lipid peroxidation ([(βControl-Hot - βCORT-Hot) - (βControl-Cold - βCORT-Cold)]: mean = 0.125, pMCMC =0.344). However, targeted contrasts showed that lipid peroxidation was lower in CORT-Hot compared to CORT-Cold lizards (βCORT-Hot - βCORT-Cold: mean = -0.242, pMCMC < 0.05).

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| Fig 2— Estimates of mitochondrial density (A, C) and metabolic capacity (B, D) in the olfactory bulbs (A, B) and optic tecta (C, D) of L. delicata hatchlings as a function of the different prenatal conditions. Black dots indicate the posterior mean, and the bars represent the SD of the estimates. The y-axis represents the posterior estimates of the variable of interest, and the x-axis represents the different prenatal conditions. Lines with asterisks represent significant differences between groups based on pMCMC values (pMCMC < 0.05), no lines indicate no significant differences. |

*Optic tecta*: We did not find significant effects of CORT, temperature, or their interaction on mitochondrial density, metabolic capacity, or ROS production in the optic tecta, neither of CORT or incubation temperature on DNA damage or lipid peroxidation (see [Fig. 2](#fig-results_energy), [Fig. 3](#fig-results_oxidative), and Table S2). However, the interaction of CORT and temperature have significant effects on DNA damage ([(βControl-Hot - βCORT-Hot) - (βControl-Cold - βCORT-Cold)]: mean = -0.211, pMCMC < 0.05), and lipid peroxidation ([(βControl-Hot - βCORT-Hot) - (βControl-Cold - βCORT-Cold)]: mean = 0.217, pMCMC < 0.05) in the optic tecta. CORT elevations had no effect on hot-incubated lizards (βControl-Hot - βCORT-Hot: mean = 0.026, pMCMC =0.611), but it decreased total DNA damage in cold-incubated animals (βControl-Cold - βCORT-Cold: mean = 0.237, pMCMC < 0.05) (see [Fig. 3](#fig-results_oxidative) C, E). The direction of the effects of CORT on lipid peroxidation were the opposite on eggs incubated at high temperatures (βControl-Hot - βCORT-Hot: mean = 0.102, pMCMC =0.188) than at low temperatures (βControl-Cold - βCORT-Cold: mean = -0.115, pMCMC =0.121), with significant differences between CORT-Hot and CORT-Cold lizards (βCORT-Hot - βCORT-Cold: mean = -0.193, pMCMC < 0.05; see [Fig. 3](#fig-results_oxidative) C). In addition, we found that females had higher DNA damage levels than males, and lipid peroxidation increased with age (see Table S4).

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| Fig 3— Estimates of ROS (A, D), DNA damage (B, E), and lipid peroxidation (C, F) in the olfactory bulbs (A - C) and optic tecta (D - F) of L. delicata hatchlings as a function of the different prenatal conditions. Black dots indicate the posterior mean, and the bars represent the SD of the estimates. The y-axis represents the posterior estimates of the variable of interest, and the x-axis represents the different prenatal conditions. Lines with asterisks represent significant differences between groups based on pMCMC values (pMCMC < 0.05), no lines indicate no significant differences. |

#### Do prenatal CORT and incubation temperature affect prey detection?

Models explained between 26 to 29.3 % of the variation in prey detection (see Table S1).

*Chemical stimulus*: Lizards detected chemical stimulus faster when exposed to prenatal CORT (βControl - βCORT: mean = 0.237, pMCMC < 0.05), but there was no effect of temperature or the interaction between CORT and temperature ([Fig. 4](#fig-results_behaviour) A and Table S2).

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| Fig 4— Estimates of detection latency of chemical (A) and visual (B) stimulus by L. delicata hatchlings as a function of the different prenatal conditions. Black dots indicate the posterior mean, and the bars represent the SD of the estimates. The y-axis represents the posterior estimates of the variable of interest, and the x-axis represents the different prenatal conditions. Lines with asterisks represent significant differences between groups based on pMCMC values (pMCMC < 0.05), no lines indicate no significant differences. |

*Visual stimulus*: There were no significant effects of CORT, temperature, or their interaction on the detection latency of visual stimuli ([Fig. 4](#fig-results_behaviour) B and Table S2).

#### Do metabolic function and oxidative stress compromise prey detection?

*Chemical perception (Olfactory bulbs)*: We found a positive effect of mitochondrial density on ROS production (mean β~Mit density~ - ROS = 0.864, pMCMC =0.080). However, there were no significant relationships between mitochondrial function or oxidative stress on prey detection, nor between other any other variables (see [Fig. 5](#fig-sem_results_OB) and Table S5).

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| Fig 5— Structural Equation Models for OB/Chemical stimulus |

*Visual perception (Optic tecta)*: We found no significant relationships within mitochondrial variables or between mitochondrial physiology and cognitive performance (see [Fig. 6](#fig-sem_results_OT) and Table S6).

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| Fig 6— Structural Equation Models for OT/Visual stimulus |

## Discussion

We examined the impact of prenatal temperature and CORT on visual and chemical perception to test whether metabolic function underpinned perception in lizards, as previously shown in mammals [[36](#ref-chaudhari2022early),[37](#ref-hara_presynaptic_2014)]. Overall, metabolic processes appeared largely resilient to prenatal conditions. However, early-life conditions produced region- and context-dependent effects on oxidative damage and prey detection. We observed interactive effects of prenatal CORT and incubation temperature on lipid peroxidation in the two brain regions examined, while the interaction affected DNA damage only in the optic tecta. Behaviourally, prenatal CORT exposure enhanced detection of chemical but not visual prey stimuli. Yet, we found no evidence that metabolism explained behavioural variation in prey detection behaviours.

#### Metabolic processes in multiple brain regions are robust to early life conditions

Contrary to our predictions, we found no significant effects of early environmental conditions on mitochondrial density, metabolic capacity, or ROS production in the optic tecta or the olfactory bulbs. These results were consistent when metabolic capacity and ROS production were standardised by mitochondrial density. Our findings suggest that metabolic function in the brain of *L. delicata* is relatively resilient to early-life conditions. While we did not observe effects in the optic tecta and olfactory bulbs, evidence from other species indicates that the effects of prenatal CORT and temperature can be region-specific. For example, incubation temperature alters metabolic capacity in some, but not all, brain nuclei of leopard geckos (*Eublepharis macularius*) [[38](#ref-coomber_independent_1997)], and prenatal stress reduces mitochondrial efficiency in select nuclei of rats (*Rattus norvegicus*) [[26](#ref-gong_chronic_2011)]. Thus, while our results point to robustness in the regions studied, other brain areas in *L. delicata* may be more sensitive to prenatal CORT and temperature.

Alternatively, the effects of early environmental conditions on metabolic function and ROS production may be present during incubation, but diminish or dissapear over time. Mitochondria are highly responsive to environmental changes and energetic demands [[28](#ref-gyllenhammer2020developmental)], and any alterations present during incubation may have dissipated once all animals developed under the same post-hatching environment. In our experiment, lizards were housed in standardized conditions prior to euthanasia at 238-356 days post-hatching (mean = 273.25). It is possible that the effects of prenatal CORT and temperature exposure had lessened by the time metabolic function and ROS production were measured. Nonetheless, even temporary changes in metabolic function during earlier stages would still be expected to affect oxidative damage, as these processes are cumulative and can lead to long-term consequences [[51](#ref-rice2002brain),[52](#ref-terman2006oxidative)].

#### Oxidative stress brought about by experiencing early life CORT are region- and temperature- dependent

We found that DNA damage and lipid peroxidation were affected in region-specific ways. Early conditions did not significantly influence DNA damage in the olfactory bulbs, but they did alter lipid peroxidation in the olfactory bulbs and the optic tecta. In the optic tecta, prenatal CORT reduced DNA damage in cold-incubated lizards but had no effect in hot-incubated animals. In both regions, CORT also interacted with incubation temperature to influence lipid peroxidation. Lizards treated with prenatal CORT and incubated at cold temperatures had higher levels of lipid peroxidation compared to the lizards from the CORT-Hot group. These contrasting patterns suggest that prenatal CORT and temperature may shape oxidative damage through molecule-specific protective pathways, potentially by upregulating different antioxidant systems. For example, prenatal stress in rats increases Cu/Zn superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity in the brain while catalase (CAT) was unaffected [[53](#ref-mcintosh1998glucocorticoids)]. Similarly, in the soft-shelled turtle (*Pelodiscus sinensis*), CAT expression elevates with increasing temperatures as turtles emerge from hibernation, whereas GPx activity remains stable [[54](#ref-tang2021antioxidant)]. Such differential regulation of antioxidant systems could explain why CORT reduces DNA damage under cold incubation but reduces lipid peroxidation under hot incubation.

However, other mechanisms could be contributing to our results. For example, CORT mobilises energy resources to facilitate physiological adjustments to stress and environmental challenges [[29](#ref-sapolsky_how_2000)]. Under cold incubation, when metabolic rates are expected to be lower, energy may be allocated towards antioxidant activity or DNA repair [[55](#ref-kawamura1991glucocorticoid),but see [53](#ref-mcintosh1998glucocorticoids)]. In contrast, at warmer temperatures, increased metabolic demands may limit the resources available for such protective processes [see [56](#ref-kim2019carry)]. These context-dependent changes may explain lower DNA damage in the optic tecta of CORT-Cold lizards, but they do not fully account for the reduced lipid peroxidation in CORT-Hot animals. Additional factors, such as temperature-dependent differences in membrane lipid composition [[57](#ref-zeis2019temperature)], may further modulate molecule-specific sensitivity to oxidative stress. For example, lipid peroxidation levels have been shown to increase in cold-acclimated *Daphnia magna* likely because of higher concentration of polyunsaturated fatty acids, which are more susceptible to oxidative damage [[57](#ref-zeis2019temperature)]. Future studies should examine the effects of prenatal CORT and temperature on metabolic rate, antioxidant regulation, and molecule structure and composition in differen brain nuclei of reptiles to parse between these hypotheses.

#### Prenatal CORT exposure enhances chemical but not visual prey detection

Detection latency decreased with CORT exposure when animals were presented with chemical, but not visual, stimuli. This effect was not related to alterations in mitochondrial physiology in the olfactory bulbs. Instead, prenatal CORT could influence other mechanisms involved in chemoreception. For instance, in rats (*R. norvegicus*), dexamethsone (a synthetic GC) enhances the ability to detect chemical stimuli by upregulating gene expression in the olfactory mucosa [[58](#ref-meunier2020olfactory)]. If similar GC-dependent transcriptional changes occur in lizards,they could alter chemosensory sensitivity independent of mitochondrial function.

Alternatively, the observed differences in chemical perception may be driven by treatment effects on motivation. We fasted lizards in our experiment for two days prior to behavioural assays to standardize hunger levels. However, the experimental treatments could have affected factors related to the animals’ internal state that we did not measure that influenced motivation. For example, prenatal CORT can increase hunger through resource mobilisation [[59](#ref-spencer2008post),[60](#ref-cossin-sevrin_effect_2022),but see [5](#ref-crino_corticosterone_2014-learn)]. This could have led to increased motivation in CORT-treated lizards decreasing their time to respond to prey chemical stimuli. Furthermore, the absence of CORT-related effects on visual perception could occur because actively foraging lizards often prioritize chemosensory cues [[61](#ref-verwaijen2007relationships)], so changes in motivation may selectively affect chemical perception. Further studies need to be conducted to understand how CORT can change foraging strategies in lizards and how this can affect their perception abilities.

#### Mitochondrial function and oxidative stress do not affect detection abilities

Mitochondria are predicted to impact cognitive performance by providing energy for neural activity or by regulating oxidative stress [[17](#ref-siegel1994basic)–[23](#ref-alexandrov_neuronal_2022)]. For example, reduced cytochrome c oxidase (COX) activity in dopaminergic neurons of the olfactory bulb of mice (*Mus musculus*) is associated with impaired olfactory capacities [[62](#ref-pass2020impact)], and links between brain metabolic function and visual acuity have also been reported in other species [reviewed in [63](#ref-wong2010energy)]. However, metabolic function did not explain variation in prey detection in *L. delicata*. In contrast, our results suggests that metabolic function is not a limiting factor for prey detection in *L. delicata*, or that *L. delicata* can sustain this cognitive function despite fluctuations in energy availability.

Similarly, oxidative stress did not influence the ability of lizards to detect visual or chemical stimuli from prey despite finding that oxidative damage varied across brain regions. Oxidative damage can decrease cognitive abilities through neuron death or senescense [[4](#ref-zhu_prenatal_2004),[18](#ref-du_dynamic_2009),[25](#ref-finkel_oxidants_2000)–[27](#ref-hoffmann_mitochondrion_2018)]. However, our Structural Equation Models showed no relationship between lipid peroxidation or DNA damage and prey perception abilities. Possibly, the levels of oxidative damage observed in our study were not sufficient to impair prey detection either because of compensatory mechanisms-such as antioxidant activity-that counteracted the negative effects of oxidative stress, or because the brain regions we studied had a high neural density that provided functional resilience [[51](#ref-rice2002brain),[64](#ref-amiel_smart_2011)]. Alternatively, the cognitive effects of oxidative stress may not be detectable at the age when we tested lizards. Oxidative damage accumulates over time, and its effects on cognition can pronounce with age [[37](#ref-hara_presynaptic_2014),[52](#ref-terman2006oxidative)]. As such, we could find strong relationships between oxidative damage and perception if older individuals were tested. In fact, we saw a significant effect of age on DNA damage in olfactory bulbs, and on lipid peroxidation in both regions. Cross-sectional studies across life stages may help reveal how oxidative damage shapes cognitive function over time.

#### Conclusions

Prenatal CORT exposure and incubation temperature produced region-specific effects in the brain of *L. delicata*. While mitochondrial function remained remarkably resilient to prenatal conditions, oxidative damage and behavioural responses varied depending on treatment and brain region. The interaction between prenatal CORT exposure and incubation temperature influenced DNA damage and lipid peroxidation in the olfactory bulbs and the optic tecta. However, these effects were not related to the perception of visual or chemical prey stimuli. Notably, chemical perception was affected by prenatal CORT alone, highlighting a selective sensitivity of perceptual abilities independent of measured brain physiology. Our findings suggest that differences in prey detection may result from treatment effects on other factors, like motivation. Future studies should explore how the environment influences mitochondrial function accross tissues, as well as the mechanisms underlying the brain’s resilience to early-life conditions.

### Ethics

Both the breeding animals and the experimental lizards were provided humane laboratory housing, with thermoregulatory opportunities, light (UV and heat) and moderate levels of humidity. Euthanasia was performed by intraperitoneal injection of a 10 mg/kg of a 10 mg/mL alfaxan solution (a potent anesthetic) followed by decapitation. We monitored the animals to ensure there was no irritation from the agent as indicated by distressed animals. Before disposing of the lizard, we confirmed the absence of righting response and pinching reflex in one of the front limbs. All the protocols complied with Australian law and were approved by the Australian National University Animal Experimentation Ethics Committee (A2022/33).

### Data accessibility

All data, data description, and R code are available in public repository [https://github.com/Pablo-Recio/CORT-Temp\_PreyD](https://github.com/Pablo-Recio/CORT_Temp_PreyD).

### Declaration of AI use

We declare Chat GPT was used for inspecting the code for errors when necessary. All other parts of the manuscript were written by the authors.

### Authors’ contributions

P.R.: conceptualization, methodology, data collection, data curation, formal analysis, writing—original draft, writing—review and editing; D.C.L.: conceptualization, methodology, data collection, data curation, writing—review and editing; O.C.: conceptualization, methodology, writing—review and editing; C.F.: conceptualization, methodology, funding acquisition, writing—review and editing; D.N.: conceptualization, methodology, funding acquisition, project administration, resources, supervision, writing—review and editing.  
All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

### Conflict of interest declaration

We declare we have no competing interests.

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