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Project title:

Sexually dimorphic brain regions in geckos: linkage between incubation temperature, sex hormones and behaviour

Host:

Henry B. John-Alder, Professor and Chair
Department of Ecology, Evolution, & Natural Resources
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Purpose of the visit:

The aim of my stay was to explore the effect of temperature during development (including embryonic stage) on sexually dimorphic brain nuclei in *Paroedura picta*, a gecko with genotypic sex determination (GSD). Such effect was described in *Eublepharis macularius*, a gecko with temperature sex determination (TSD), where studied brain nuclei depended on incubation temperature instead of gonadal sex (Coomber et al. 1997).

Background:

In vertebrates, the most critical regions of brain for male- and female-typical sexual behaviour are the preoptic area (POA) and ventromedial nucleus of hypothalamus (VMN), respectively. In lizards, the POA is required for male-typical mounting and intromission behaviour, and the size of this brain region is generally larger in males than in females. In contrast, the VMN is required for female-typical receptivity and is relatively larger in females (Crews et al. 1990). It is generally accepted that sexual dimorphism in the size of these brain regions is controlled by levels of gonadal sex steroids (e.g. Adkins-Regan 2005). However, in *E. macularius,* differences in the volumes of POA and VMN are not determined by gonadal sex, but are set by temperature during incubation (Coomber et al. 1997).

In contrast to *E. macularius*, *P. picta* is characterized by GSD, not TSD (Blumberg et al. 2002). To test the role of temperature on brain morphology in *P. picta*, animals used for brain analyses were raised at the constant temperature 27°C (including embryonic stage), which produces the largest sexual dimorphism in body size in this species (Starostová et al. 2010). Moreover, we also tested the role of hormones on the POA and VMN via surgical manipulation gonadal steroids. Treatment groups included control intact males, castrated males, castrated males provided with testosterone (T) implants, control intact females, females allowed to reproduce regularly, females with elevated levels of T and ovarectomised females. Surgery was performed on animals that were sexually mature but not fully grown. After reaching final body size the influence of treatment on animal behaviour (either courtship or aggressive) was tested. Finally, six months after surgery, all individuals were sacrificed and their tissues stored at -80° C for further analyses.

The aim of the project, conducted in the physiological laboratory of Prof. Henry John-Alder, was to investigate the volumes of sexually dimorphic brain areas (POA and VMN) of *P. picta* in 1) intact control individuals to determine the extent of sexual differences at given temperature, and in 2) individuals with manipulated hormonal levels to test whether volume is affected by the treatment.

Description of the work carried out during the visit:

After my arrival at Rutgers University and prior to the experiment, I learned how to embed frozen brain tissue, slice sections using a cryostat (Ames Cryostat II with Tissue-Tek disposable blade) and stain sliced sections in the physiological laboratory of Prof. Henry John-Alder.

Laboratory procedures and data analyses:

Brains stored at -80° C were transfer to cryostat set at -18° C. After embedding in O.C.T. Compound (Tissue-Tek), tissue was sectioned in the coronal plane at 20 µm. Every fourth section was stained with thionin (stain for neurons and glia) and fixed. Images of stained tissue were obtained using a camera attached to a light microscope (Nikon Eclipse E800 light microscope; digital camera Nikon DXM 1200F) with 20 fold magnification. Brain nuclei including the POA, VMH and *habenula* (a sexually monomorphic nuclei used as control) were located with the aid of the stereotaxic brain atlases for the *Gecko gecko* (Smeets et al. 1986) and *Anolis carolinensis* (Greenberg 1982). The procedure adapted from Coomber et al. (1997) was followed. Obtained photos were analysed by ImageJ software (ImageJ 1.44p; National Institutes of Health, USA) that measures the area of traced brain regions (Fig 1.). Measurements were only made on the left side of the brain to minimize error due to plane of section. The mathematical formula adapted from Crews et al. (1990) (which can be adjusted when thickness between measured sections varies) was used to calculate volume of the given brain region of each individual.

The size of brain regions may be affected by individual differences in overall brain size, thus the volume of measured nuclei are presented relative to total brain volume. The overall brain area was measured from the start of the POA to the end of the VMN to calculate the volume of the forebrain in the same manner as the individual brain regions. Volumes of the POA, VMN and *habenula* were then divided by volume of this forebrain region. To reach VMN (the most distal nuclei measured), each brain was sliced into approximately 400 slices (from the beginning of telencephalon to the end of diencephalon), therefore analyses of 30 brains (five to four brains per treatment group) is presented here as preliminary results. However, more brains were sectioned and stained. Completing the measurements will add to our findings so our final analysis will be more robust.

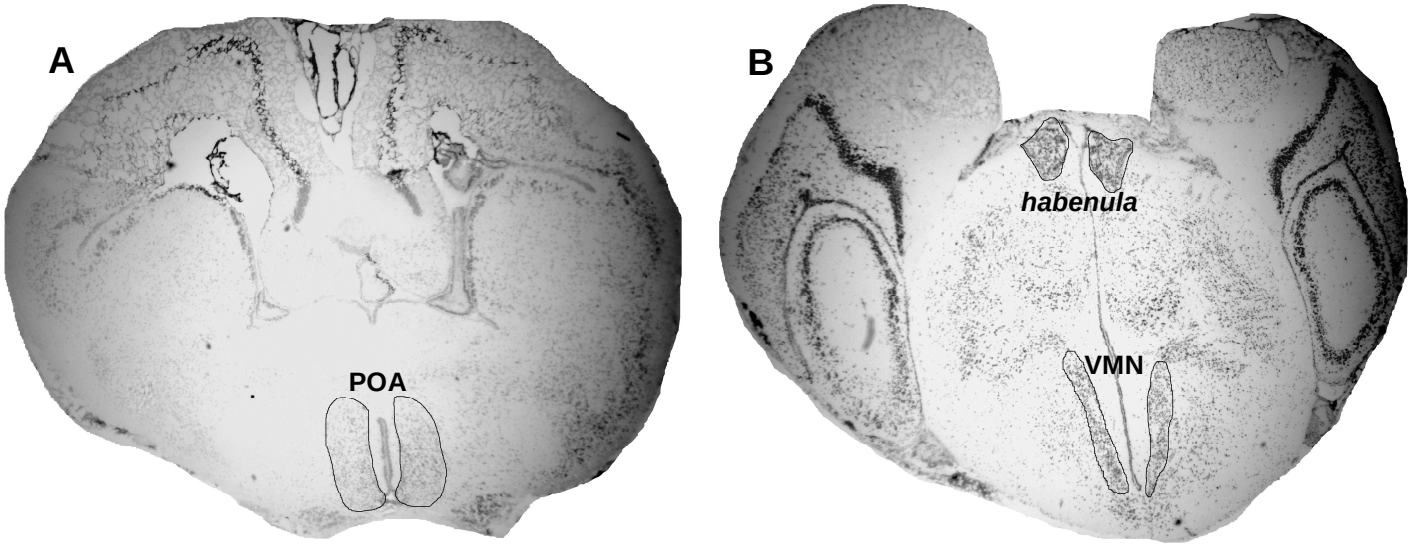

Fig. 1. POA is located in the upper part of forebrain (**A**), whereas VMN appears when *habenula* is ending in the most distant part of forebrain (**B**). Individual areas are highlighted.

Description of the main results obtained:

Based on the preliminary results, none of the measured brain nuclei are sexually dimorphic in *P.picta.* Using nonparametric Kruskal-Wallis ANOVA, we found no differences in relative volume of habenula ($H = 9.92$, $p = 0.128$), POA ($H = 2.7$, $p = 0.845$) or the VMN ($H = 12.51$, $p = 0.052$) among treatment groups. Although not statistically significant, a marginal effect of treatment on volume of the VMN is seen in Fig. 2, where mated and T treated females tend to have larger VMN than other groups but both intact males and intact females seem to have similar VMN.

Nevertheless, these preliminary results suggest that gonadal sex of *P. picta* does not affect the volume of brain regions that are commonly sexually dimorphic among vertebrates. Furthermore, hormonal manipulations in sexually mature animals do not affect the volume of the POA or VMN. Perhaps the effect of temperature during development of the POA and VMN is strong in *P. picta*, a gecko with GSD, which is surprisingly similar to *E. macularius*, a gecko with TSD.

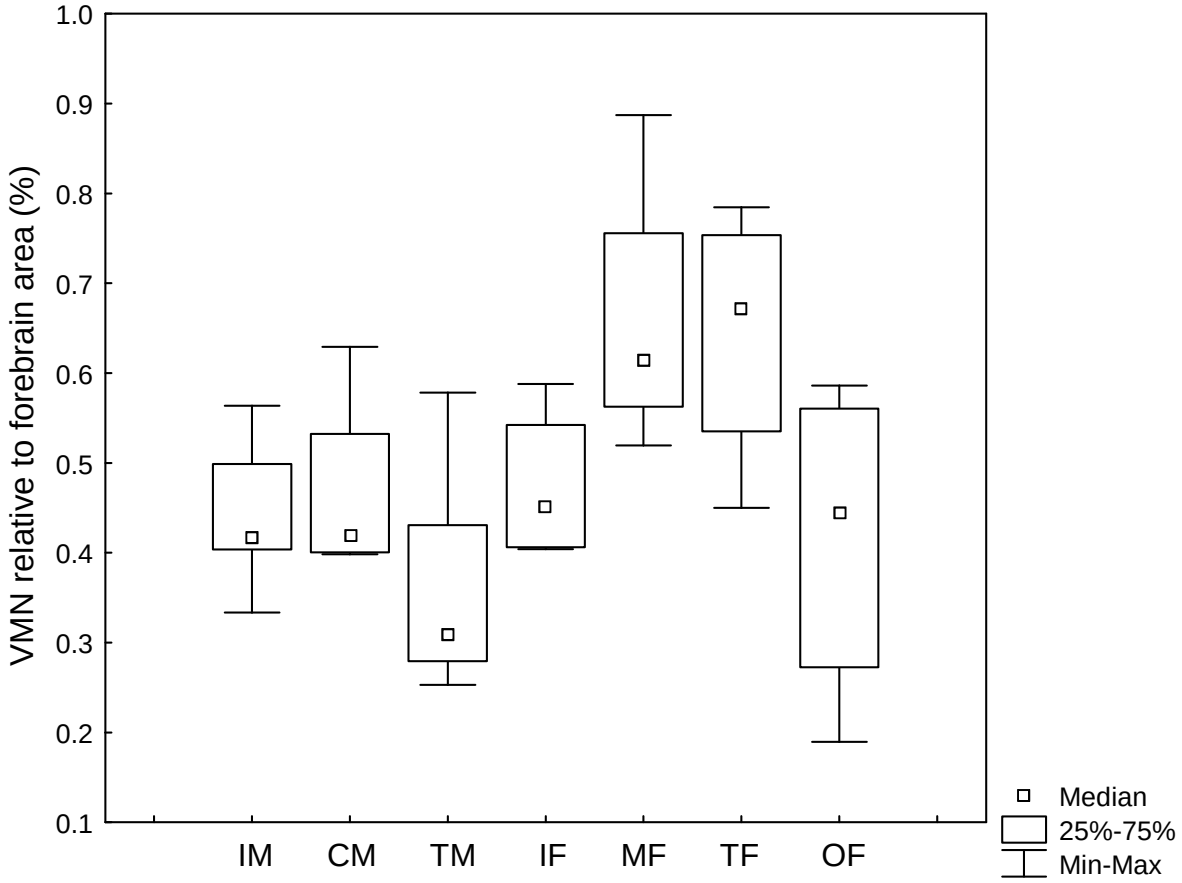

Fig. 2. Differences among treatment groups in volume of VMN relative to forebrain area. Mated females and T treated females tend to have larger relative volume. Abbreviation IM is for intact males, CM for castrated males, TM for T treated castrates, IF for intact females, MF for mated females, TF for T treated females and OF for ovarectomised females.

List of planned publications and planned further collaboration:

We plan to collaborate on writing at least two publications. One will focus on the sexually dimorphic brain regions in the context of incubation temperature, androgens and behaviour in *P. picta*. The second publication will compare these results from *P. picta* with results from other geckos with GSD that were treated with similar hormonal manipulations.

References:

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