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Short communication

Short communication: Baroreflex function in embryonic emus (*Dromiceius novaehollandiae*)

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ABSTRACT

The baroreflex involves cardiovascular homeostatic mechanisms that buffer the system against acute deviations in arterial blood pressure. It is comprised of the cardiac limb which involves adjustments in heart rate and the peripheral limb which involves adjustments in vascular resistance. This negative feedback loop mechanism has been investigated in numerous species of adult vertebrates, however our understanding of the maturation and functional importance of the reflex in developing animals remains poorly understood. In egglaying species, our knowledge of this mechanism is limited to the domestic chicken embryo and the embryonic alligator. While each of these species possess a cardiac baroreflex prior to hatching, they differ in the timing when it becomes functional, with the embryonic chicken possessing the reflex at 90% of incubation, while the alligator possesses the reflex at 70% of incubation. In an effort to determine if bird species might share similar patterns of active baroreflex function, we studied embryonic emus (*Dromiceius novaehollandiae).* However, we hypothesized that emus would possess a pattern of baroreflex function similar to that of the American alligator given the emu embryo possesses functional vagal tone at 70% of incubation, possibly indicating a more mature collection of cardiovascular control mechanism than those found in embryonic chickens. Our findings illustrate that emu embryos possess a hypotensive baroreflex at 90% of incubation. Therefore, our data fail to support our original hypothesis. While only two species of birds have been studied in this context, it could indicate that baroreflex function is not essential for cardiovascular homeostasis in birds for the majority of in ovo development.

The baroreflex is a physiological mechanism that helps regulate arterial blood pressure and maintain cardiovascular homeostasis. It operates through a negative feedback loop, primarily involving specialized sensors (baroreceptors) located typically in the arterial blood vessel walls. These baroreceptors detect increases in arterial blood pressure, then send neural impulses to the brain's medulla oblongata, which acts as the physiological control center. In response, the medulla oblongata triggers a series of adjustments to decrease heart rate, dilate blood vessels, and reduce blood volume, all of which act to return blood pressure back to normal levels. Conversely, if blood pressure drops, the baroreflex increases heart rate and blood vessel resistance, raising arterial blood pressure. This intricate system, the general features of which have been described in all vertebrates, plays a crucial role in maintaining stable arterial blood pressure (Hughes et al., 2023; Karemaker and Wesseling, 2008; Kaufmann et al., 2020). During embryonic development, the baroreflex mechanism may play a role in adjustments to deviations in arterial pressure that could impact cardiovascular function at some point in the growing embryo/fetus (Crossley II et al., 2003b; Elfwing et al., 2011; Segar, 1997). These baroreceptors can play a role in regulating the fetal heart rate and blood pressure, in concert with other regulatory mechanisms, ensuring steady blood flow to support the developing organs and tissues (Crossley II et al., 2003b; Segar et al., 1992). Any disruption or malfunction in the baroreflex during this critical phase can lead to cardiovascular complications and developmental abnormalities (Giussani et al., 2003; Kaufmann et al., 2020; Skeffington et al., 2020). Therefore, the baroreflex mechanism not only maintains stability in the maternal cardiovascular system but may also do so in the developing animal.

Despite the baroreflex's role in maintaining cardiovascular function and overall homeostasis in vertebrates, relatively few comparative

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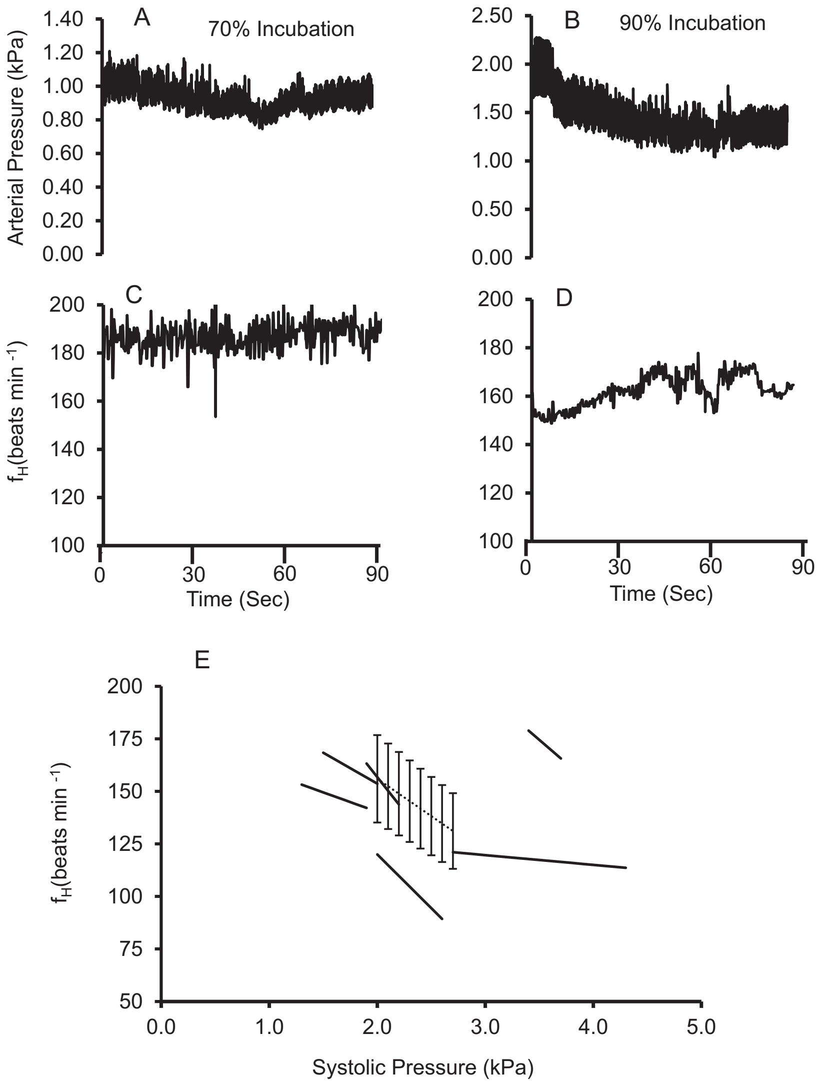

Fig. 1. Representative traces of the arterial pressure (A, B) and heart rate (f_H) response (C, D) of individual emu embryos to an injection of 50 μg kg^{-1} of the vasodilator sodium nitroprusside, injected at time 0. Data are from individual embryos at 70% (A,C) or 90% (B,D) of incubation. Panel E contains a compilation of lines generated from the linear regression equation from either individual animals (solid lines) or an average (dashed line with standard errors) based on data from all 6 animals at 90% of incubation. A clear inverse relationship exists between systolic pressure and f_H.

studies have investigated the ontogeny of baroreflex function. The cardiac limb of the baroreflex becomes functional prior to hatching in the embryonic domestic chicken, *Gallus gallus domesticus* (Altimiras and Crossley II, 2000; Mueller et al., 2013) and the embryonic American alligator, *Alligator mississippiensis* (Crossley II et al., 2003; Mueller et al., 2018; Nelson et al., 2018). However, the timing of the onset of baroreflex function in these species differs, with the embryonic chicken developing baroreflex function after 90% of incubation while the American alligator possesses a baroreflex cardiac limb at 70% of incubation (Crossley II et al., 2003).

A critical component of the baroreflex's cardiac limb is activation or withdrawal of vagal stimulation on heart rate (Moore et al., 2022; Studinger et al., 2007). Interestingly, tonic or continuous vagal suppression of heart rate in chickens and alligators is absent throughout the majority of development (Crossley II and Altimiras, 2000; Crossley II et al., 2003). To determine if functional vagal tone is coupled to a functional cardiac baroreflex in embryonic egg-laying terrestrial vertebrates, we studied embryos of the emu, *Dromiceius novaehollandiae*, a ratite bird related to ostriches, cassowaries and rheas. Emu embryos show vagal tone on heart rate as early as 70% of incubation (Crossley II et al., 2003a). We hypothesized that embryonic emus would possess a hypertensive cardiac baroreflex, which equates to a decrease in heart rate as arterial pressure increases. In addition, we expected the hypertensive reflex would be present at 70% of development and increase in strength corresponding to the increasing effect of vagal tone on heart rate in embryonic emus.

Eggs of the emu, *Dromiceius novaehollandiae,* were collected at the Cross Timbers Emu Ranch (Flower Mound, Texas). All eggs ($n = 18$) were then transported to the University of North Texas and placed in an incubator (G.Q.F. Model 1536E) at 36 °C with a relative humidity of 30% - the incubation conditions recommended for emu embryos. Eggs were studied on days 35, and 45 post fertilization, which corresponded to 70% ($n = 8$) and 90% ($n = 10$) of the typically 50-day incubation period.

For surgery, eggs were placed in a water-jacketed temperature control chamber set at 36 °C which was maintain with a circulating waterbath (VWR International, LLC, West Chester, PA, USA). A 2 cm square window was cut in the shell to expose vessels of the underlying chorioallantoic membrane (CAM). A tertiary CAM artery was occlusively catheterized using heat-pulled polyethylene tubing (PE-90) filled with heparinized (100 unit ml^{-1}) saline (0.9%) (Crossley II and Altimiras, 2000).

Following arterial catheterization, eggs were placed in one of four

Table 1

Baroreflex parameters for embryonic emus at 90% of incubation. Calculations were made based on a linear regression model to determine the slope of the linear portion of the curve (beats min^{-1} kPa^{-1}), R^2 is the correlation coefficient between f_H and each P parameter, Min is the minimal *P* value in response to the injection of sodium nitroprusside (SNP), Max is the P value prior to the injection of SNP, Gain is the gain of the baroreflex and $\text{Gain}_{\text{Norm}}$ is the normalized gain of the baroreflex. Cardiac baroreflex function analysis was conducted on the six of 10 embryos that exhibited an inverse relationship between P and f_H. The four non-responsive embryos were excluded. Data are presented as mean values± SEM.

Variable	Arterial pressure			
	Systolic	Diastolic	Mean	Pulse
Slope (beats min^{-1} kPa^{-1})	−35.48 ± 9.06	−61.53 ± 16.69	−47.19 ± 12.35	59.04 ± 15.35
R^2	0.69 ± 0.05	0.56 ± 0.10	0.60 ± 0.07	0.59 ± 0.08
Max f_H(beat min^{-1})	153 ± 10			
Min f_H(beat min^{-1})	134 ± 13			
Gain (beats min^{-1} kPa^{-1})	35.48 ± 9.06	61.53 ± 16.69	47.19 ± 12.35	59.04 ± 15.35
$\text{Gain}_{\text{Norm}}$ (Unitless)	0.71 ± 0.22	0.87 ± 0.38	0.78 ± 0.29	0.42 ± 0.12
Min (kPa)	2.1 ± 0.3	1.4 ± 0.2	1.8 ± 0.3	0.7 ± 0.1
Max (kPa)	2.7 ± 0.4	1.8 ± 0.3	2.2 ± 0.3	1.0 ± 0.2

1200 ml temperature-controlled chamber fitted with a lid with ports for externalization of catheters and tubes for air flow. The catheter was then passed through a port and connected to a pressure transducer (World Percision Instruments, Inc. type BLPR, Sarasota, FL USA) attached to a bridge amplifier (CB Sciences model ETH-400, Milford, MA USA). Humidified 36 °C air was continuously circulated into each experimental chamber during the period of study at a rate of 1.5 l min^{-1}. A data acquisitions system (Powerlab, ADinstruments Colorado Springs, CO, USA) was used to collect the output signal at a sampling frequency of 100 Hz. After the embryos were placed in their experimental chamber, they were monitored for 1 h to allow cardiovascular values to stabilize. Prior to experimental manipulations, control baseline data were collected from each embryo for 30–60 min. After this control period, the cardiac limb of the baroreflex was investigated by serially injecting first the vasodilator sodium nitroprusside at doses of 25 and 50 μg kg^{-1} to induce hypotension. This was followed by injections of the vasoconstrictor phenylephrine at doses of 30, 60 and 120 μg kg^{-1} to induce hypertension. Between each drug injection, arterial pressure parameters (P) and heart rate (f_H) were allowed to return to pre-injection values. Baroreflex analysis was conducted on a section of the data trace in which arterial pressure increased or decreased and f_H inversely decreased or increased was selected for analysis. Beat-to-beat changes within this range were visualized by plotting arterial blood pressure parameters (kPa) against f_H (beat min^{-1}) in the pressure module of LabChart Pro 8 (ADInstruments). In all cases analysis was conducted using systolic pressure (P_{Sys}), diastolic pressure (P_{Dia}), mean arterial pressure (P_M) and pulse pressure (P_{Pulse}) as the independent variables. f_H verses pressure parameters were analyzed using a linear regression model of Statistica 12 (StatSoft, Tulsa, OK, USA) as previously conducted in a study of chicken embryos (Altimiras and Crossley II, 2000). Baroreflex gain was calculated as −1 multiplied by the slope of the regression for Df_H/DP.

For comparison to prior studies of other species, gain was normalized to control P and f_H as $\text{Gain}_{\text{Norm}} = \text{Gain} * (P/f_H)$ and expressed as a percentage change in f_H per percentage change in P (Altimiras and Crossley 2000). These analyses were conducted using systolic, diastolic, mean arterial and pulse pressure as the independent variables, with values of gain calculated for all parameters.

Somewhat surprisingly, given previous demonstrations of cardiac vagal tone, both the hypotensive and hypertensive baroreflex were absent in emu embryos at 70% of incubation (Fig. 1A and C). Only at 90% of incubation was an inverse relationship between P and f_H evident in response to one dose of the vasodilator sodium nitroprusside in 6 of 10 embryos studied (Fig. 1B and D). Thus, the onset of a functional baroreflex was limited to a specific narrow point in late development, as is also the case for embryonic chickens (Altimiras and Crossley II, 2000). Further, the reflex in emus was only evident in response to decreases in arterial pressure, or a hypotensive baroreflex (Fig. 1D). This differs from embryonic American alligators, where only a hypertensive baroreflex has been identified (Crossley II et al., 2003b). In emus, the greatest correlation between measured variables was between f_H and systolic arterial pressure (average $R^2 = 0.69 \pm 0.05$), (Table 1) compared to the other arterial pressure parameters used as independent variables. In addition, given the reflex was only functional at 90% of development, we could not make comparisons to other time points of incubation in this species. However, a comparison of the baroreflex parameters between embryonic chickens and emus at similar points of incubation (90% of incubation) revealed differences between the two species (Altimiras and Crossley II, 2000). Specifically, the slope or sensitivity of the cardiac baroreflex was approximately 50% greater in the emu compared to the chicken (Table 1) (Alitimiras and Crossley 2000) when P_M was used as the independent variable in the linear regression. Further, when the gain of the baroreflex was normalized for control f_H and P_M, at approximately 90% of incubation the values for the species were markedly diffferent (chickens = 0.22 vs emus =0.78) (Altimrias and Crossley 2000) (Table 1). Most notably, even though vagal tone is active on f_H in embryonic emus by 70% and is greatest at 90% of incubation (Crossley II et al., 2003a) functional cardiac baroreflex function was nonetheless absent until 90% of incubation in the emus (Fig. 1A and B). Importantly, the peripheral limb of the baroreflex, or the vascular response, was not assessed in the current or in prior studies of embryonic egg laying species. The peripheral limb may be a major contributor to the overall baroreflex function as previously suggested for the chicken (Altimiras and Crossley II, 2000). In the other embryonic bird species investigated for baroreflex function, the embryonic chicken, the reflex is functional by 90% of incubation, but it is not correlated with the onset of vagal tone (Crossley II and Altimiras, 2000). Vagal tone is also absent in embryonic alligators prior to hatch even though they possess a hypertensive baroreflex that rapidly resets (Eme et al., 2011). In the case of embryonic chickens, it has been suggested that the onset of a functional baroreflex may be dependent on factors that include a threshold for arterial pressure (Altimiras and Crossley II, 2000). Arterial pressure of embryonic emus progressively increases with incubation, so there may also be a pressure threshold for the activation of both a hypertensive and a hypotensive baroreflex in emus. Interestingly, while vagal tone on f_H is present in embryonic emus at 70% of incubation, the cardiac limb of the baroreflex did not appear before 90% of incubation (Fig. 1B and D). Thus, our hypothesis that the baroreflex will coincide with the onset of vagal tone is rejected. As suggested for embryonic chickens, our findings call into question the importance of a cardiac limb baroreflex function for maintaining cardiovascular homeostasis during embryonic development in emus. As noted earlier, there are very few comparative studies of the development of baroreflexes in non-mammals, and studies on the embryos/larvae of additional species is highly warranted.

CRediT authorship contribution statement

Dane A. Crossley: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Brian P. Bagatto:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Ed M. Dzialowski:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Warren W. Burggren:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Conceptualization. **James W. Hicks:** Writing – review & editing, Writing – original draft, 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.

Data availability

Data will be made available on request.

References

Altimiras, J., Crossley II, D.A., 2000. Control of blood pressure mediated by baroreflex changes of heart rate in the chicken embryo (*Gallus gallus*). Am. J. Phys. Regul. Integr. Comp. Phys. 278, R980–R986.

Crossley II, D.A., Altimiras, J., 2000. Ontogeny of cholinergic and adrenergic cardiovascular regulation in the domestic chicken (*Gallus gallus*). Am. J. Physiol. Regul. Integr. Comp. Physiol. 279, R1091–R1098.

Crossley II, D.A., Hicks, J.W., Altimiras, J., 2003. Ontogeny of baroreflex control in the American alligator *alligator mississippiensis*. J. Exp. Biol. 206, 2895–2902.

Crossley II, D.A., Bagatto, B.P., Dzialowski, E.M., Burggren, W.W., 2003a. Maturation of cardiovascular control mechanisms in the embryonic emu (Dromiceius novaehollandiae). J. Exp. Biol. 206, 2703–2710.

Crossley II, D.A., Hicks, J.W., Altimiras, J., 2003b. Ontogeny of baroreflex control in the American alligator *alligator mississippiensis*. J. Exp. Biol. 206, 2895–2902.

Elfwing, M., Lundengard, K., Altimiras, J., 2011. Fetal development of baroreflex sensitivity: the chicken embryo as a case model. Respir. Physiol. Neurobiol. 178, 75–83.

Eme, J., Altimiras, J., Hicks, J.W., Crossley II, D.A., 2011. Hypoxic alligator embryos: chronic hypoxia, catecholamine levels and autonomic responses of in ovo alligators. Comp. Biochem. Physiol. A- Mol. Integrat. Physiol. 160, 412–420.

Giussani, D.A., Forhead, A.J., Gardner, D.S., Fletcher, A.J., Allen, W.R., Fowden, A.L., 2003. Postnatal cardiovascular function after manipulation of fetal growth by embryo transfer in the horse. J. Physiol. 547, 67–76.

Hughes, G.W., Moore, J.P., Lord, R.N., 2023. Barosensory vessel mechanics and the vascular sympathetic baroreflex: impact on blood pressure homeostasis. Exp. Physiol. 108, 1245–1249.

Karemaker, J.M., Wesseling, K.H., 2008. Variability in cardiovascular control: the baroreflex reconsidered. Cardiovasc. Eng. 8, 23–29.

Kaufmann, H., Norcliffe-Kaufmann, L., Palma, J.A., 2020. Baroreflex dysfunction. N. Engl. J. Med. 382, 163–178.

Moore, J.P., Simpson, L.L., Drinkhill, M.J., 2022. Differential contributions of cardiac, coronary and pulmonary artery vagal mechanoreceptors to reflex control of the circulation. J. Physiol. 600, 4069–4087.

Mueller, C.A., Burggren, W.W., Crossley II, D.A., 2013. ANG II and baroreflex control of heart rate in embryonic chickens (Gallus gallus domesticus). Am. J. Physiol.-Reg. I. 305, R855–R863.

Mueller, C.A., Eme, J., Tate, K.B., Crossley II, D.A., 2018. Chronic captopril treatment reveals the role of ANG II in cardiovascular function of embryonic American alligators (Alligator mississippiensis). J. Comp. Physiol. B. 188, 657–669.

Nelson, D., Crossley II, D.A., Elsey, R.M., Tate, K.B., 2018. Cardiovascular adjustments with egg temperature at 90% incubation in embryonic American alligators, Alligator mississippiensis. J. Comp. Physiol. B. 188, 471–480.

Segar, J.L., 1997. Ontogeny of the arterial and cardiopulmonary baroreflex during fetal and postnatal life. Am. J. Phys. 273, R457–R471.

Segar, J.L., Hajduczok, G., Smith, B.A., Merrill, D.C., Robillard, J.E., 1992. Ontogeny of baroreflex control of renal sympathetic nerve activity and heart rate. Am. J. Phys. 263, H1819–H1826.

Skeffington, K.L., Beck, C., Itani, N., Niu, Y., Shaw, C.J., Giussani, D.A., 2020. Hypertension programmed in adult hens by isolated effects of developmental hypoxia in Ovo. Hypertension 76, 533–544.

Studinger, P., Goldstein, R., Taylor, J.A., 2007. Mechanical and neural contributions to hysteresis in the cardiac vagal limb of the arterial baroreflex. J. Physiol. 583, 1041–1048.