Effects of Δ9-Tetrahydrocannabinol on Cardiovascular Function in Chicken Embryos

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Introduction

- The recent legalization of Marijuana has led to an increase in use, including by pregnant women[1].
- Studies in humans and animals suggest that the psychogenic component of marijuana, Δ9-THC, may alter birthweight and may affect the developing cardiovascular system[2].
- The long-term goal of this study is to create a chick embryo model to determine the effects of Δ9-THC on an embryo, independent of any effects on Δ9-THC on the physiology of the mother or the function of the placenta.

Methods

- Ten fertilized eggs from domestic hens (*Gallus gallus*) were used in the study (IACUC Protocol #19.030).
- The incubation was performed in an incubator R-com 50 pro at a temperature of 37.0±0.5 °C and humidity of 60±5%. The eggs were rotated (90^0) in automatic mode once every 60 minutes.
- Eggs were injected with 50 µl warm saline on alternating days from embryonic day 2 until embryonic day 20.
- The Buddy Mk2 Digital Egg Monitor was used to record heart rate of the chicken embryos *in ovo* (**Figure 1**). The Buddy Egg Monitor uses 3 LED's attached to a sensor pad to detect the heart rate of the embryo.
- To determine basal heart rate and any changes in basal heart rate during the last week of gestation, heart rate was measured on embryonic days 13, 15, and 17. Heart rate was recorded every 30 seconds for a total of 10 minutes and a mean value for each egg calculated.
- To determine the response of the embryonic heart to a physiological challenge, we measured the change in heart rate that occurred at low ambient temperature.
- Heart rate was recorded every 30 seconds for 10 minutes when the eggs were inside the incubator at 37°C.
- Later in the day, the same eggs were moved to room temperature (25°C) and heart rate recorded again every 30 seconds for 10 minutes (**Figure 3**).
- Data are presented as mean ± standard error of the mean (SEM).

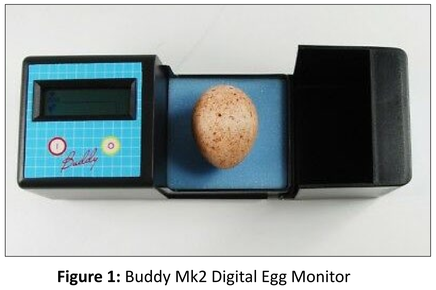

Figure 1: Buddy Mk2 Digital Egg Monitor

Results

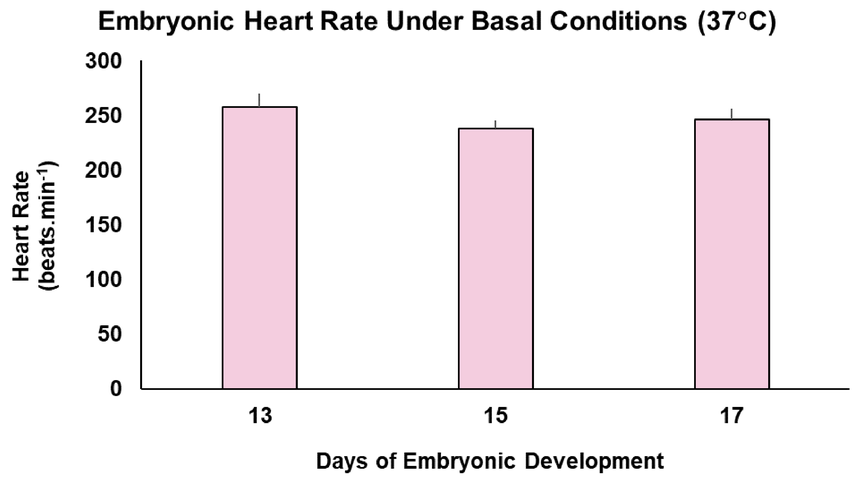

Figure 2: Mean heart rate in chicken embryos (n=10) on embryonic days 13, 15, and 17. Data are mean ± SEM. *P<0.05.

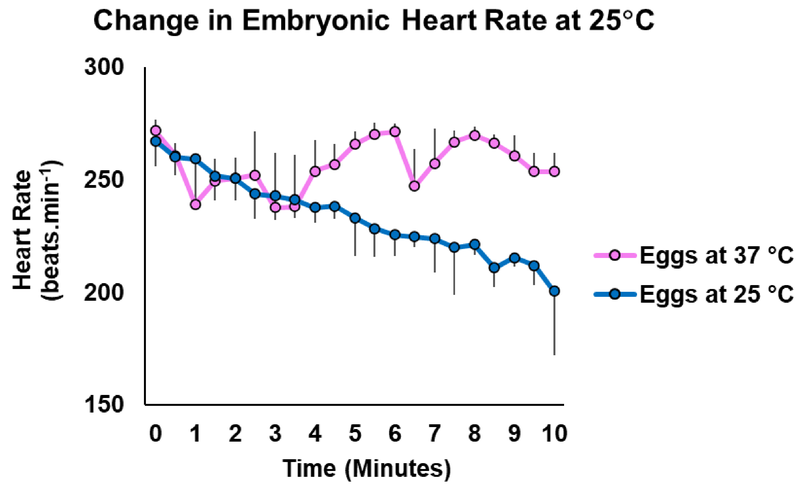

Figure 3: Heart rate response to control (37°C; n=10) or low ambient temperature (25°C; n=10) in chicken embryos on embryonic day 18. Data are mean ± SEM.

Results

- Heart rate was similar on days 13, 15 and 17 of embryonic development (**Figure 2**).
- At the onset of the experiment to test the effects of ambient temperature on heart rate over time, heart rate was similar in both groups (**Figure 3**).
- Heart rate in chicken embryos at 37°C varied around 250 beats.min^{-1} consistent with the mean heart rate measured on days 13, 15, and 17 (**Figures 2 and 3**).
- Heart rate in chicken embryos that were moved to an ambient temperature of 25°C decreased over time (**Figure 3**).
- After 10 minutes, heart rate was ~ 50 beats.min^{-1} lower in chick embryos at low ambient temperature than heart rate in chick embryos at 37° (**Figure 3**).

Conclusion

The current study demonstrated that basal embryonic heart rate and the embryonic heart rate response to a physiological challenge can be measured in a chicken embryo *in ovo*.

Future Directions

- Further studies will use the chick embryo model to determine the effects of Δ9-THC on the developing cardiovascular system.
- The chick embryo model has the potential to provide insight into the direct effects of Δ9-THC on the heart independent of confounding changes in maternal/placental physiology.

References

1. Warner TD, Roussos-Ross D, Behnke M. It's not your mother's marijuana: effects on maternal-fetal health and the developing child. Clin Perinatol. 2014 Dec;41(4):877-94.

2. Benevenuto SG, Domenico MD, Martins MA *et al*. Recreational use of marijuana during pregnancy and negative gestational and fetal outcomes: An experimental study in mice. Toxicology. 2017 Feb 1;376:94-101.

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