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Review

Genetic, environmental and maternal influences on embryonic cardiac rhythms

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Abstract

The relative roles of an animal's genetic constituents and environmental factors in influencing physiological variables such has heart rate have not been extensively investigated. This paper considers how heart rate patterns in the developing animal can be regulated, and how a combination of 'nature' and 'nurture' may interact to produce discrete patterns of heart rate change during development. The concept of the 'developmental trajectory' is evoked to generate a conceptual framework for how physiological development can be perturbed by environmental factors. Data are provided from three species showing how 'clutch-effects' (the fact that siblings perform physiologically much more similarly than non-siblings) can greatly influence the variance observed when collecting data on heart rate during development. Finally, so-called 'maternal effects', which are the influences on embryos of environmental experiences of the parents, are discussed as potentially confounding effects in the study of the genetic basis for physiological patterns of change during development.

Keywords: Development; Embryo; Genetic control; Maternal effect; Heart rate

1. Introduction

The role of 'nature' (i.e. genetic constituents) versus 'nurture' (i.e. environmental influences) has been hotly debated in many contexts, most notably as determinants of human behavior as revealed by so-called 'separated twin studies'. The same interpretive dichotomy exists for physiological processes, with the interacting role of genes versus environment being intensively studied as causative agents in cardiovascular disease, for example (see Refs. [8,13]). Certainly, both genetic and environmental constituents are likely to influence physiological processes in the early embryo, but the relative contributions of each to the metabolism, heart rate, or growth of embryos has not been extensively explored. The objective of this paper is to review some of the data relating to possible genetic and environmental influences on the cardiac rhythms of vertebrate embryos. A longer-term goal will be to move to quantitative genetic approaches to ascertain why we observe the often complex yet predictable heart rate patterns that unfold during embryonic development.

2. The concept of 'developmental trajectory'

A concept proving useful in thinking about development and its potential perturbation is that of the 'developmental trajectory' [3,5]. Consider the analogy of a developing embryo like a missile on its launch pad. Just as the flight path of the missile is pre-programmed by a consideration of the ballistic properties of the missile, the developmental events of the embryo are pre-programmed by genetic instructions. After the missile lifts off of its launch pad, the pre-programmed flight path is impacted by a variety of variables (e.g. the amount of fuel in the missile, the density of the air through which it passes, prevailing winds) that can subtley or profoundly alter its trajectory en route to its target. Simi-

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larly, the fertilized egg of an animal is launched on a genetically dictated developmental trajectory that can be perturbed by a number of factors, both abiotic (environmental temperature, oxygenation, pH) and biotic (nutritional reserves, predation, competition). Like the missile deflected en route from its targeted landing site to a new site, the development of the embryo can be modified en route to its pre-determined adult phenotype, with the end result being a phenotype that varies from that dictated genetically stricto sensu.

The analogy of the developing embryo as an in-flight missile allows us to understand in rather straight-forward terms how abiotic and biotic factors can influence the developmental trajectories of the whole organism. Is this analogy extendable to the consideration of individual organ systems? That is, could individual organ systems follow distinctly separate developmental trajectories in response to a particular environmental perturbation. As a hypothetical example, consider the effects of hypoxia on embryonic tissues. Certainly, many cell and tissue types are sensitive to hypoxia. However, we might pose and then test the hypothesize that hypoxia would more likely create non-lethal (and possible beneficial) changes in the developmental trajectory of those systems more intimately involved in gas exchange (e.g. respiratory, cardiovascular systems), because changes in these systems might offer the opportunity of 'rescue' from the deleterious effects of hypoxia. Thus, one could imagine (or, more importantly, actually document) the differing trajectories of two different systems — one more sensitive to hypoxia-as dictated by prevailing environmental conditions. Limited supporting evidence arises from aspects of lung size and blood characteristics of developing anurans exposed to chronic hypoxia [6,14,15]. Separate trajectories seem apparent for blood cellular/biochemical characteristics and for gas exchange organ structure during chronic hypoxic exposure in the bullfrog, *Rana catesbeiana*. The blood properties of early larvae are quite fixed, with neither hypoxia or hyperoxia having any influence on their development. This is in sharp contrast to juveniles/adults, where hypoxia causes large increases in hematocrit, hemoglobin concentration, and erythrocyte concentration and a sharp decrease in blood P_{50}. However, the surface area and blood-water or blood-air barrier of the skin and lungs and gills of tadpoles is highly labile, responding with increased area and decreased diffusion distance as oxygen availability declines. This differs greatly from the situation in juveniles/adults, where lung and skin structure is unaffected by chronic hypoxic exposure. Future experiments clearly determining the linkage, or lack thereof, of developmental trajectories for separate organ systems will be interesting, indeed.

3. Heart rate developmental trajectories: influence of genes and environment

The concept of developmental trajectory is broadly applicable to physiological processes such as heart rate. Indeed, it provides a conceptual framework for asking questions, generating hypotheses and testing them. A question that our laboratory has recently attempted to answer is "Do genes play a role in regulating specific heart rate patterns (and variation in them) during early development?" The first clues of the putative strong genetic effects came from studies of heart rate during development in altricial birds [17]. The intent of the original study was to determine the overall (i.e. mean) pattern of heart rate change in embryos during development, focusing on the altricial species the bank swallow (*Riparia riparia*) and pigeons (*Columba domestica*) both domesticated and wild. 'Conventional' analysis of these data (i.e. generation of mean values representing overall species performance) revealed that heart rate showed a specific pattern of change as development progressed from 13 days before external pipping to external pipping. These patterns of heart changes, based on the mean values produced by in some cases the averaging of dozens of individual embryos, permitted Tazawa et al. [17] to offer novel observations on the similarities and differences between heart rate pattern development in altricial compared with the much better known precocial birds. However, a re-analysis of these data that preserved the individual identity of each embryo through time, revealed that there was a highly significant 'clutch effect'. That is, the pattern of heart rate change during development was significantly more similar in siblings (embryos from the same clutch of eggs) than in non-siblings (Fig. 1) in both bank swallows and pigeons. Importantly, all data for a single species was collected on multiple clutches of eggs all incubated and measured over the same period of time in the same incubator, thus minimizing if not eliminating environmental perturbations that could have differentially modified some but not other developmental trajectories for heart rate.

Having serendipitously discovered this 'clutch effect' influencing heart rate in bird embryos, we set out to determine whether other vertebrates that laid clutches of eggs similarly showed this phenomenon. Experiments were performed on multiple egg clutches of the Puerto rican cave coqui, *Eleutherodactylus cookii* collected after laying in the wild but maintained and monitored in the laboratory [4]. Rather than laying 2–5 eggs in a clutch, like the altricial birds measured earlier, the cave coqui lays as many as 40 eggs in a clutch. Consequently, fairly subtle heart rate effects attributable to clutch effects could be statistically teased apart from other factors contributing to heart rate variation. The variation about the mean for all data was miniscule,

and a distinct pattern of increasing heart rate during development for the species could readily be discerned. However, when mean values for *individual clutches* were plotted against time, it becomes clear that there are distinct differences between clutches in heart rate at any given point in development. Again, as for altricial bird embryos, eleuthyrid anuran frogs show a strong clutch effect, in which intraclutch variation is far smaller than interclutch variation.

As a final example, D. Crossley (unpublished) working in our laboratory has determined heart rate during development in the African brown house snake (*Lamprophis fuliginosus*). Once again, intraclutch variation in heart rate — in this instance over a much longer 60 day period of development — is much smaller than interclutch heart rate.

Collectively these data from three very disparate groups — altricial birds, anuran amphibians, and colubrid snakes — indicate that heart rate changes during development are more similar in siblings than non-siblings. Moreover, the effect is not small or insignificant. A sibling group of embryos that may collectively have the highest heart rates at, say, 50% of the way through the embryonic development period may, in fact, revert to having the lowest mean heart rate 60% of the way to hatching.

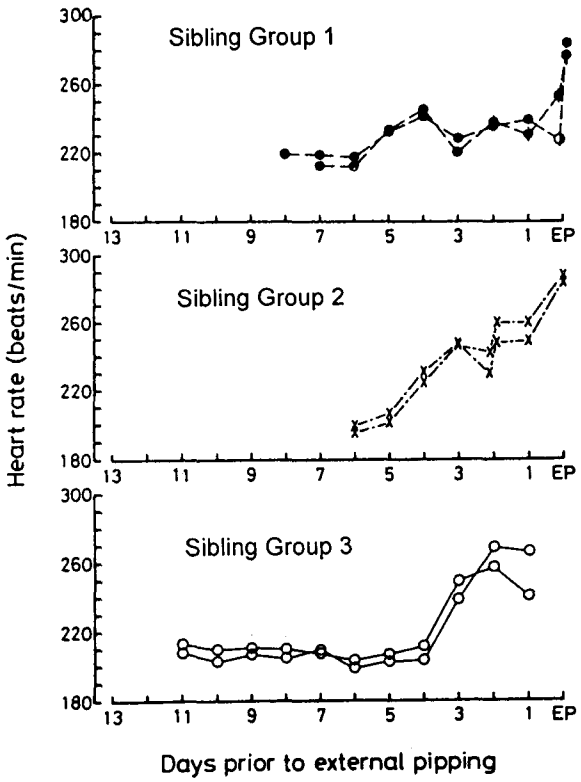

Fig. 1. Intraclutch differences in heart rate changes during development in the altricial domestic pigeon, *Columba domestica*. Each panel shows the mean daily heart rate in sibling pairs of embryos. Note that while the general pattern of sharply increasing heart rate in the final days of embryonic development holds for all three sibling groups, the day-to-day variance is much less when comparing sibs than when comparing non-subs. (From Ref. [7]).

How do we explain these 'clutch' or 'sibling' effects? Changing environmental conditions influencing the embryo during the incubation period appears not a viable explanation for heart rates that may be tens of beats higher in certain clutches at certain times, for two reasons:

1. all three data sets (bird, frog and snake embryos) involved collection of heart rate data for each embryo under extremely similar, if not identical experimental conditions, with careful attention paid to temperature, oxygenation, photoperiod, mechanical or other forms of disturbance, etc.;
2. even when quite large changes in environment (e.g. hypoxia or hyperoxia, mechanical disturbance) are experimentally induced in bird, frog or snake embryos, usually little or no change in measured heart rate results. This relative difficulty in producing cardiac chronotropic effects holds for the very late stages where regulatory mechanisms are in place and operational, and is even more evident in those relatively early embryonic stages with immature or as yet undeveloped regulatory mechanisms where large sibling effects nonetheless have already become evident. In those cases where ecologically relevant environmentally relevant stimuli do have heart rate effects, the changes in heart rate are often nowhere near as striking as those observed to exist between clutches measured under strictly controlled conditions.

The clutch effect observed in the absence of environmental variation in our experiments appears to be most easily explained by the expression of genetic regulation of embryonic cardiovascular physiology. That developmental milestones in physiological processes are genetically regulated in the embryo is, of course, to be expected. Most certainly the general tendency for heart rate to increase more sharply in the final stages of embryonic development in altricial species of birds is a product of the genetic control of the expression of proteins that directly or indirectly affect the form and function of the heart's pacemaker cells. However, our data additionally suggest that rate variations superimposed on the general pattern of heart rate change may also genetically dictated.

Returning to our analogy of a missile following a trajectory, a missile's general trajectory can be controlled by the programmed flight plan at launch, but additional course corrections (perhaps to navigate a missile around a mountain) can also be pre-programmed at launch to occur only at specific times during the missile's flight. So, too, the simplest explanation for the clutch (sibling) effect in vertebrate heart rate is that there are genetically determined rather than

environmentally stimulated adjustments in heart rate occurring during development.

Apart from ruling on the basis of circumstantial evidence that environmental influences are 'highly unlikely' in affecting heart rate trajectory, is it possible to experimentally verify the important role of genetics? To pursue this question, we have sought a more tractable animal model system in the form of the nine banded armadillo, *Dasypus novemcinctus*. Common across the American South, this animal is notable for two reasons. First, it is the only animal model for human leprosy, which is endemic in its natural populations, and which adds intriguing challenges to safely maintaining them in captivity. Second, (and the reason we studied them), they exhibit a phenomenon known as polyembryony. The developing blastocyst invariably buds off into four embryos, each of which develops independently to term [12]. Thus, a litter always consists of four genetically identical individuals. Polyembryony in the armadillo presents an ideal opportunity to explore the nature/nurture argument within mammals. Environmental differences can never be completely eliminated during development in mammals, because there are regional differences within the placenta in terms of blood flow and tissue proliferation, and thus in nutrient delivery and waste removal to each of the embryos. However, on the day of birth, one can assume that the genetic regulation of physiological processes is greatest, and the environmental component the smallest, as at any other time during the subsequent life of the animal. By comparing between- and within-litter variation at birth, we have discerned that genetically identical siblings have heart rates at birth that are more similar than unrelated neonates (B. Bagatto, D. Crossley and W. Burggren, unpublished). Preliminary analysis of data from these studies suggest that genetic influences are indeed prominent in determining not only heart rate, but also oxygen consumption and ventilation frequency between birth and day 8.

4. Maternal effects as an alternate explanation for the 'clutch effect'

A genetic regulation of both general trends and specific daily values for heart rate in developing vertebrate embryos appears to be the most parsimonious explanation of our data. Are there other possible viable explanations beside environmental influences which cannot be excluded by our experimental design? We cannot discard so-called *maternal effects* — an effect on an embryo that is caused by a process or behavior carried out by the mother that influences the subsequent developmental trajectory of her offspring (see Ref. [1] for a more detailed definition and literature review). Maternal effects appear to be poorly understood (when even recognized!) within the comparative physiological community. While possible maternal effects are frequently overlooked as a source of data among developing animals, most biologists are well aware of their effects. For example, when a human mother consumes excessive amounts of alcohol during the critical window for central nervous system development in her fetus (typically between week 3 and 16), her child may exhibit mental retardation — a well-known syndrome called Fetal Alcohol Syndrome. Another example can be found in the experiments of Sinervo et al. [16] on so-called 'allometric engineering' in lizards, where they showed that experimental removal of yolk from lizard eggs resulted in a proportionally smaller hatchling lizard. Thus, by extension to natural situations, mothers who produced larger eggs with more yolk would presumably produced larger offspring, with presumably a greater chance for survival. As a final example, raptors exposed to DDT produce thinner egg shells. While the breakage of the egg is an obvious deleterious effect, a egg shell that is thinner but that can still hold its integrity through hatching may also produce both greater gas exchange and excessive water vapor loss, all of which could have highly complex, interactive effects on the physiology and morphology of the developing embryo. Additional examples of various categories of maternal affects are provided by Lombardi [10]. Indeed, an excellent source for much additional information on maternal effects is American Zoologist, vol. 36(2), 1996, which presents the proceedings of a Society for Integrative and Comparative Biology Symposium entitled "Maternal Effects on Early Life History: Their Persistence and Impact on Organismal Ecology".

Maternal effects can be far-reaching and profound, yet subtle. As Bernardo [1] comments, "Maternal effects contribute complexity to phenotypes, as well as to biologists' attempts to analzye phenotypes". Given the potential for insidious maternal effects influencing physiological events as they unfold in the developing embryo, how can we differentiate between genetic and maternal effects? Experimental protocols can and have been designed to tease apart genetic and environmental influences. Such protocols typically require extensive numbers of animals and breedings over several generations (e.g. Ref. [2]). To our knowledge, there have not yet been any attempts to determine whether maternal effects could influence heart rate (or other physiological variables) in vertebrates.

5. Conclusions

Emerging data from studies on a variety of vertebrates suggest that developmental changes in heart rate are likely to be precisely genetically regulated across

time, yet also can be subject to large alterations when their embryonic environment is perturbed. Our current experimental approach for sorting out the relative roles of genetic and environmental effects depends upon unusual life histories to minimize genetic variation. While these preliminary probes of the 'nature/nurture' components of physiological phenotype have been productive, they are subject to obfuscation by strong 'clutch effects', in which genetically more similar individual embryos show more similar patterns of heart rate change through development. Such clutch effects are likely as a result of the common genetic background of related individuals, but could also reflect so-called 'maternal effects'. In light of these various contributing factors, interpretation of variation in both existing physiological data and that coming from future studies will most efficiently be conducted by using the emerging tools for partitioning variance into genetic and environmental components as advocated by Refs [9,11]). With a more complete understanding of the sources of physiological variance, we will better understand the genetic underpinnings of complex physiological changes during development.

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