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*J Mol Cell Cardiol*. Author manuscript; available in PMC 2013 July 30.

Published in final edited form as:
*J Mol Cell Cardiol*. 2012 May ; 52(5): 921–922. doi:10.1016/j.yjmcc.2012.01.014.

# Une cellule type?

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The function $f(x)=\exp\left(-(x-\mu)^2/2\sigma^2\right)/\left(\sigma\sqrt{2\pi}\right)$ is called the Gaussian distribution or normal distribution with about equal frequency. We have used the two names interchangeably. However, these names reflect the two fundamentally different interpretations of $f(x)$. Karl Frederick Gauss (1777–1855) recognized that $f(x)$could describe the probability distribution of errors in the measurement of a *single* entity, say an angle of a star or that of a geodetic triangle [1]. Adolphe Quetelet (1796–1874) found that anthropometric measures such as the height of men or the girth of soldiers' chests could also be described by $f(x)$. From this *population* distribution Quetelet defined in 1844 "*un homme type*", *the average man* [2]. In Quetelet's interpretation of $f(x)$, differences from the mean $\mu$ were not errors but reflected the natural variation in the population. $f(x)$ quantifies what we mean by *normal*. "Deviation" in standard deviation ($\sigma$) connotes no malevolence but "deviant" has no such neutrality.

In studying biological systems, most experimentalists probably do not have the luxury of measuring a single entity multiple times. Instead we measure the $Ca^{2+}$ current (say) in one myocyte, usually killing the cell in the process and move on to another myocyte. The distribution of $Ca^{2+}$ currents we get is then like Quetelet's distribution of heights, a description of the variability of the $Ca^{2+}$ currents in a population of cells. Yet, we suspect, that for many researchers the first interpretation (and perhaps only interpretation) of the measured distribution is that of a distribution of errors. We cast no stones. For a long time we viewed the deviations from the *mean* to result from measurement errors: subtle temperature fluctuations, differences in seal resistance, incomplete capacitance compensation, etc. The fault was not in the cells but in ourselves.

Viewed from this error perspective our measurements were like the pale shadows cast on Plato's cave from which we were to infer the ideal value. And what is the ideal value? Assuming that errors are unbiased and random, then the mean would be the most probable estimate of the true value. But notice that we have fallen back to a Gaussian interpretation of the error distribution of measuring a single—in this case, ideal—cell.

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**Disclosures**
None declared.

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We doubt anyone consciously subscribes to the notion of a Platonic ideal cell but we do unwittingly use the ideal cell when we use the mean value—and only the mean value—in our computational models. The idea of an ideal cell is valid if (1) the inherent cell-to-cell variability is small and (2) if the variability has only small functional consequence. Now we face the new challenges to determine how variable the cardiac myocytes are and how significantly these variations affect cardiac dynamics and function.

## 1. Cell-to-cell variability

Cell functions are carried out by proteins so differences in protein levels between cells should give rise to differences in cell behavior. In the past decade remarkable strides have been made in measuring the cell-to-cell variability in protein levels using flow cytometry [3,4] or automated microscopy [5]. In one study, the concentrations of more than 2500 GFP-tagged proteins were measured in individual cells [4]. For many proteins, the concentration distribution follows a *lognormal* distribution with coefficients of variation (CV=mean/standard deviation) ranging from ~0.1 to ~0.6 [3-6]. The lognormal distribution is known as a heavy-tailed distribution in contrast to the normal distribution, which is a thin-tailed distribution [7]. In a heavy-tailed distribution, values above the mean by many standard deviations can still have a significant chance of occurring, whichwould be virtually impossible in a normal (Gaussian) distribution. Functionally such a broad range of protein concentrations could account for large cell-to-cell variation in the time between exposure to TRAIL (tumor necrosis factor related apoptosis-inducing ligand) and apoptosis [5].

Another indication of large cell-to-cell variability comes from our recent study in which we measured multiple $K^+$ currents ($I_{K1}$, $I_{Ks}$, $I_{Kr}$) and the nisoldipine-sensitive current ($I_{Niso}$, the sum of the L-type $Ca^{2+}$ current and the $Na^+$-$Ca^{2+}$ exchange current) in individual ventricular myocytes [8]. We found that the current density and the charge density (normalized by the cell capacitance) carried by $K^+$ currents or $I_{Niso}$ can vary ~10-fold between different cells.

In addition to studying variability along the protein concentration or electric current axes, Bass et al. [9] in this issue offers a way of studying variability along the morphological axis. In this paper Bass et al. develop algorithms for determining metrics of cell size, shape, and organization. Cell area and perimeter quantify size; elongation and circularity characterize shape; and uniformity indexes sarcomeric organization. Before these metrics can be determined, one cell must be distinguished from another. The algorithm they develop does an impressive job of defining the boundaries of neonatal myocytes that have complex shapes. Once the boundary is defined then the morphological metrics can be determined. The process of segmenting the boundary and determining the metrics is entirely automatic requiring no human intervention, which is important for three reasons. First, the automated process eliminates differences arising when different observers do these measurements manually. Second, possible bias that may be introduced when the observer is not blinded to the experimental conditions can no longer occur. Thirdly, automation increases the speed of the measurements. In their study, almost 7000 cells were analyzed in about 2 h. Large numbers of cells need to be measured in order to assess the full range of cellular variability and the speed of automation makes these measurements practical.

Just as height and chest girth provide distinct descriptions of a man's morphology, it turns out that the different indices used by Bass et al. are distinctive markers of cell morphology. Cell area and perimeter are correlated but because of the complex geometry of the neonatal cell they are not redundant. Likewise, elongation and circularity are related but one is not necessarily superfluous. The Saucerman group exposed the neonatal myocytes to agonists that activate different hypertrophic pathways. Not surprisingly, when any of these pathwayswere activated, cell size metrics increased. More interestingly, the other metrics,

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circularity, elongation, uniformity, and cell-cell contact, were differentially altered. For example, $\alpha$-adrenergic activation is unique in causingmore uniform sarcomere spacing. Whether a subset of themetrics used by Bass et al. will prove to be sufficient to characterize themorphology of a cell orwhether the set needs to be enlarged only further studies can tell. Nevertheless, the indices used here can already provide a signature for the different activation pathways.

The nearly 7000 cells measured reveal substantial cell-to-cell variability in all of the morphological metrics. It will be of interest to extend these studies to the adult and aging myocytes to see if the variability changes with time. Variability in mRNA expression in themouse heart increases with age [10] so a concomitant increase in variability of protein expression would not be surprising.

Even isogenic cells derived from a single cell can have markedly different protein concentrations and different responses to agonists [5,11,12]. Differences in protein concentrations can arise simply from unequal protein distribution (stochastic partitioning) between daughter cells at cell division [13]. Multiple divisions give rise to a lognormal distribution. Therefore, it would not be surprising to find large cell-to-cell variability even in myocytes from the same region such as the epicardial layer. Morphological studies like those done by Saucerman's group might provide a practical way of determining how much variability occurs in cells in the same region of the heart.

Does an average cell, *une cellule type*, exist? Even from a parochial view of a single protein, one would be hard pressed to call a cell with a protein concentration equaling the population mean an average or typical cell when the population distribution is heavy-tailed like a lognormal distribution. A man of average height but possessing enormous wealth would unlikely to be called an average guy. Likewise, a cell whose $x$ protein concentration equaling the population mean $\bar{x}$ might have a $y$ protein concentration very different from $\bar{y}$. In fact, the probability that a cell will have all its protein concentrations equaling the population means ($\bar{x}; \bar{y}; \bar{z}, \ldots$) become vanishingly small as the number of different proteins increase. Thus from this viewpoint, an average cell does not exist. But if all cells are so different, how do organisms or an organ like the heart function? In a syncytium do the functional differences get damped out just as a few aberrantly firing myocytes fail to trigger an ectopic beat because of a source-sink mismatch [14]? Is there a point where variability of cells becomes large enough to affect the function of the heart? These are some of the new challenging questions that confront us as we learn more about the inherent variability of cells.

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