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Dynamic Transcriptomics: Transcriptomic Discovery of a Biological Multiple-Input Multiple-Output Heart Control Mechanism

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Abstract: We will recapitulate here a narrative in which we have just now been able to solve an important, frustrating and long standing puzzle as to how a brain control function of the heart is performed. The key to solving this puzzle has proved to be the acquisition of transcriptional profile data from single neurons. Analysis of this data forced a reconsideration of the fundamental structure of the brain control system. In turn, this reconsideration has led to subsequent re-ordering of a great deal of neuroanatomical and physiological data into a pattern that finally makes a great deal of sense.

Keywords: neuroscience, autonomic homeostasis, MIMO, single cell analysis, transcriptome, systems biology

1. INTRODUCTION

The role of the vagus nerve in controlling cardiac function is essential. Low vagal activity is a prognostic indicator of cardiac disease including heart failure, arrhythmias and sudden cardiac death – as well as coronary artery disease. As a result there is considerable interest in novel ways of targeting the cardiac vagus and the potential for therapeutics outcomes. For example, this symposium comes at a time when chronic stimulation of the vagus nerve is being trialed as a therapeutic treatment in human patients with heart failure. What controls the vagus and how its influence is so important in maintaining a healthy heart is the subject of this monograph. We will describe this function in terms of a biological controller.

A brain control mechanism adjusts the function of the heart via the vagus nerve on a beat-to-beat time scale. This mechanism is the so-called baroreceptor vagal reflex. Examining Figure 1 we observe that stretch receptors called baroreceptors are located on the major arteries (labeled CS and AA) where they sense the blood pressure as stretch of the arteries on each beat of the heart. These pressure sensors send this information to the brain via nerves (IX and X) that end on neurons in a brain structure called the Nucleus Tractus Solitarius (NTS). Following central processing of this information in the brain a control signal is sent, as seen in Figure 1, to the heart (post-ganglionic parasympathetics) via the vagus nerve (X) nerve to regulate the ensuing beat of the heart.

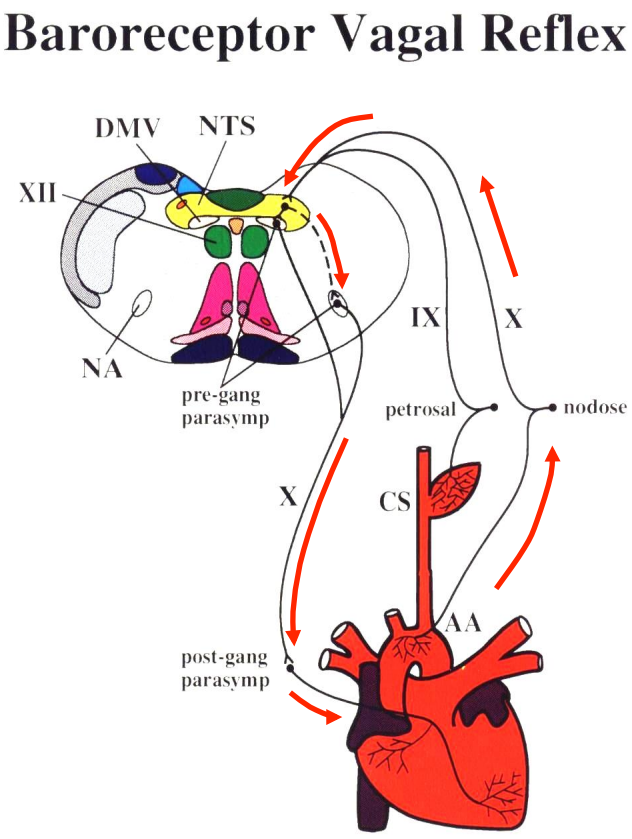

Figure 1. The anatomical organization of the baroreceptor vagal reflex.

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As we began to analyze this biological control we recognized its simplified structure was that of a single-input single-output feedback controller as shown in Figure 2, taken from Henson et al. 1994.

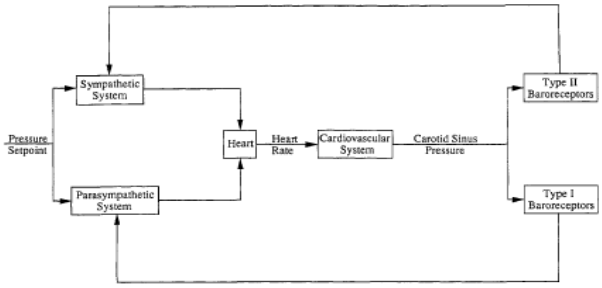

Figure 2. Simplified representation of the baroreceptor reflex for the integrated control strategy. Henson et al., 1994.

As we performed experiments on the structures of Figure 1 we aimed to understand the homeostatic regulation in terms of Figure 2.

1.1 Initial Expectancies: "How the Brain Works" and the Puzzle of Central Neuronal Physiology

Neurons have generally been thought of as "information processing" elements using a "neural code" made up of discrete voltage "spikes" in which the spike rate carries the information from neuron to neuron in a circuit. This expectation is fulfilled in the case of the first limb of the baroreflex arc, where the sensors carry reliable firing rates that that fully encode the details of each blood pressure pulse.

However, when neurophysiological studies were carried to the NTS the brain cells targeted by the sensory input failed to display firing rate behavior easily interpretable as a neural coding of the blood pressure (Rogers et al. 1993; Rogers et al. 1996). As you can see in Figure 3, the ongoing activity of these neurons is low rate and variable, with a transient derivative encoding of a rise in pressure, but no sustained change in activity encoding the elevated pressure.

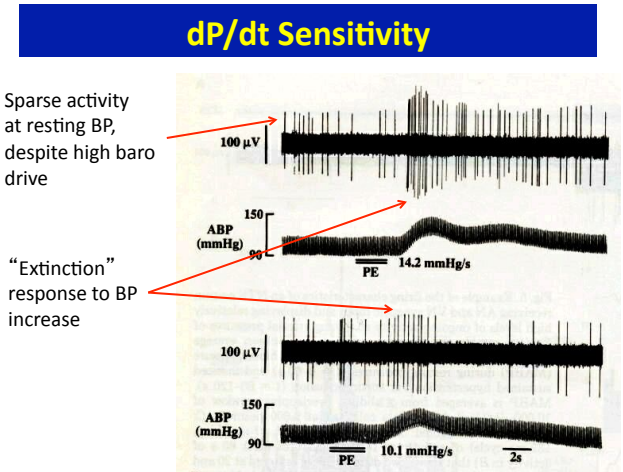

Figure 3. Recordings from two different NTS neurons that receive direct sensory baroreceptor inputs.

Since the vagal output to the heart is seen to have a cardiac rhythm, this was a disappointment and a frustrating mystery. Why and how did the powerful rhythmic input activity disappear, only to reappear in the brain's output to the heart? Further, the vagal control output to the heart is known to produce a cardiac rhythmic activity with each heart beat. How is the input and output rhythmic but the central neurons are not?

The explanation the field turned to, within the constraints of the absence of an expected rate coding, was the hypothesis that there is a large population of identical neurons producing the rate code en masse as a population code.

2. RECENT GENOMICS EXPERIMENTS AND RESULTS

2.1 A Systems Approach

In our current genomic experiments we discovered the NTS is highly heterogeneous, far from homogeneous.

We have focused on unraveling and integrating the brainstem regulatory networks at multiple scales including cell-cell interactions and intracellular processes spanning signaling, transcriptional and post-transcriptional regulation, and electrophysiology. In probing these functional networks, we need to be able to assay and manipulate molecular processes occurring in individual cells on a very large scale of sample numbers and types, in their tissue and phenotypical context, to support network reconstruction. These requirements and approaches are uncommon in present mammalian systems biology practice and require synthesis of novel technical approaches into a coherent resource. We outline our strategy and present the results from our recent high-throughput experiments and computational modeling as first steps in reaching this goal. Importantly, our analysis thus far revealed a dynamic landscape of cell phenotypes and several network processes underpinning the robustness/plasticity of function in the brainstem.

2.1 Single Cell Transcriptome

We obtained a high-dimensional single neuron gene expression data set comprised of 28,880 data points representing expression of 96 genes each in 300 single neurons lifted from the NTS of 6 rats. We selected the individual neurons based on expression of Tyrosine Hydroxylase (TH) protein as a marker for catecholaminergic phenotype, or Fos protein as an immediate early response to hypertension perturbation. NTS catecholaminergic (TH+) neurons have been shown to play a major role in controlling the blood pressure set point (Duale et al. 2007). Fos+ cells in NTS have been shown to be the second-order neurons that receive blood pressure sensory information from the arterial baroreceptors (Chan & Sawchenko 1998).

We selected the 96 genes as encompassing multiple key signaling pathways downstream of Angiotensin II Receptor Type 1 (AT1R), immediate early transcriptional regulators, and targeted neuronal functions. Our previous microarray gene expression time series data on NTS response to

phenylephrine-induced acute hypertension implicated significant differential gene expression in these networks (Khan et al. 2008). This gene set enabled us to obtain a representative snapshot of the neuronal adaptive transcriptomic state in response to hypertension perturbation.

The prevailing conceptualization of a SISO control system would necessitate identical neurons that may differ from another in a stochastic manner, with the computation occurring via a population rate code. If this formulation indeed accurately captures the NTS neuronal phenotypes, then we should expect to see an uncorrelated stochastic gene expression across single cells around a certain 'average phenotype'.

Our results reveal an unexpected organization of neuronal phenotypes that is based on a graded level of correlated gene expression across single cells (Park et al. in review). Individual neurons, rather than showing stochastic gene expression, were ordered along a gene expression gradient that separates the catecholaminergic set point control neurons at one extreme and the second-order neurons receiving baroreceptor input at the other end of the spectrum.

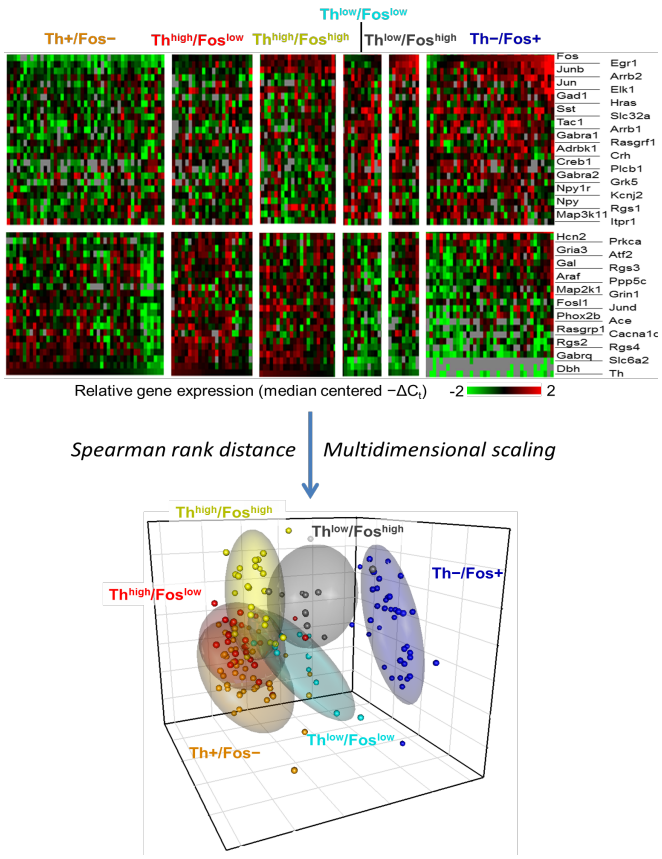

Figure 4: Single cell gene expression analysis reveals a gradient of neuronal phenotypes and co-regulated modules. Multivariate data analysis, gene expression patterns (heat map visualizing down-regulation (green), up-regulation (red), and non-differentiation (black) relative to housekeeping genes) assessed by high-throughput real-time PCR are used to compare individual neurons via statistical measures (Spearman Rank Distance). Multidimensional scaling is used to visualize multidimensional "distances" between single cells in a lower (3D) space. The group colors and clouds are based on Th and Fos gene expression levels. An overall gene expression gradient pattern can be observed in the gene expression profile of the 48 highly variable across the extreme subtypes where single cells group together into distinguishable cell states with respect to the synaptic input types.

The cell-cell differences in gene expression ('variability') were embedded within this structured organization of transcriptional regulation and imply an ordered neuronal function based on the inputs to the individual cells. If the NTS control action is determined by averaging over such population, it is not possible to provide a fine-grained control based on the physiological needs represented in the spectrum of inputs into NTS. Based on the above results, we conclude that blood pressure control action ('output') of such an input-shaped neuronal network is likely determined by which neurons along the graded continuum are active. This is consistent with a multiple-input multiple-output (MIMO) control system in which various inputs are integrated to serve appropriate physiological needs.

We hold our new conceptualization of computation in NTS as an 'input-based MIMO control code' to reinterpret the previously confusing anatomical data on the efferent arc of the baroreflex.

3.THE NEURAL CODE: FUNCTIONAL IMPLICATIONS AS TO HOW THE NTS MUST WORK

The NTS does not produce a population rate code and the reason is that the population is heterogeneous. The NTS neurons receive multiple inputs and behave so as a specific set of neurons are selected depending on the state of the many inputs to the population. Thus the neural code is a "which neurons when" code and a single spike is sufficient signal from that set of neurons on each heart beat. We were forced by the new single cell data to abandon the identical population idea and discovery of the explanation of combinatorial multiple inputs for heterogeneity.

This requires parallel lines of different kinds of cardiac control - if we revisit the literature can this be supported? Does the evidence support the possibility that the heart has several distinct kinds of control from the brain, each independently regulated by the brain?

PUTTING ALL THE PIECES TOGETHER: USING THE PARALLEL CONTROL CONCEPT TO AID IN UNDERSTANDING PRIOR LITERATURE: *Prior evidence is consistent with the concept that the control of the heart is via multiple outputs*

We have relevant prior data showing the nature of the way in which the vagus nerve innervates the heart. The cardiac vagus nerve controls the heart via postganglionic neurons at the heart itself. Our data on the nature of the innervation of the post ganglionic neurons by individual vagal axons shows each selects a distinct subset of post ganglionic neurons and innervates these with a remarkable density of input, called a

basket like ending. The implication is that there is a selective but powerful influence of each output axon. This kind of organization is consistent with the concept of parallel lines of functional control and provides and explanation and gives meaning to these results.

A montage of several composite projections of stacks of optical sections illustrating the projection fields of dorsal motor nucleus of the vagus (DmnX) axons in an atrial ganglion.

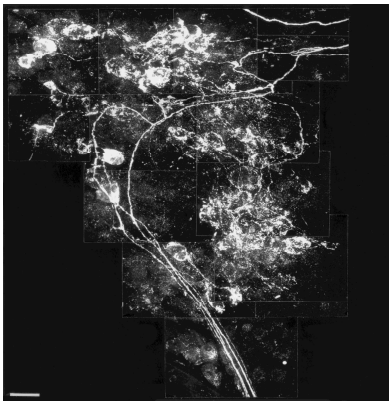

Figure 5. The vagal innervation to the heart ends with dense, selective innervation of distinct subsets of postganglionic target neurons at the heart. Cheng et al. 1999

In addition, we have relevant prior data on the diverse cardiac structures - functions that the vagus controls. Standish et al. 1994 and Standish et al. 1995 injected a viral tracer that has the property of being transported across synapses to identify the sequence of neurons forming a circuit. We injected this viral construct into specific locations at the heart to discover if they received vagal control, by finding if the tracer worked its way from the specific cardiac site back across the postganglionic neuron, then past the preganglionic neuron and finally labeling neurons in the NTS. We discovered a remarkably broad and robust innervation of: the cardiac ventricles, conduction fibers, AV and SA nodes, and the coronary arteries. These data provide a neuroanatomical correlate to the physiological influence of the vagus nerve on ventricular function, contractility, rate, rhythm of the heart. Further, these various specific injections labeled distinct subsets of NTS neurons. These findings were hard to reconcile with the prior idea that the vagus produces a single control signal, but are very compatible with the concept of parallel lines of control.

There are additional data from our own and other laboratories that are also consistent with independent lines of control that show, for example, that NTS neurons have diverse dendritic tree organization, implying that each neuron is sampling a different set of inputs to the NTS, and neurophysiological studies showing activity of specific subsets of vagal preganglionic influence specific cardiac functions.

We find that the prior anatomical and physiological literature fits together into a consistent and greatly improved explanatory structure by using the concept clears of parallel control.

4. HOW THE HEART IS CONTROLLED

Taken together, the baroreflex appears to work according to the schematic in Figure 6. Effectively:

- Distinct BP inputs find distinct targets in a cloud of unique NTS cells
- NTS cells respond depends on integrative state of all inputs
- Active cells find distinct subsets cardiovagal neurons
- Cardiovagal neurons have functionally distinct and specific projections to the heart

By this kind of mechanism, NTS coding then should look like our recordings in Figure 3:

- Implication is that control depends on *which* NTS neurons are active – rather than a population rate code
- Expect irregular, low rate activity from any individual NTS neuron with control influences

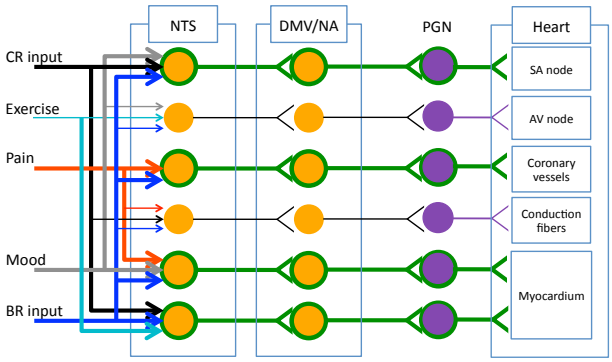

Figure 6. Schematic of a MIMO control scheme accounting for the baroreflex control of the heart. NTS neurons receive multiple inputs related to cardiorespiratory CR demands, exercise, pain, mood, and baroreceptor BR signals. NTS neurons adaptively respond to these combinatorial inputs and communicate with neurons of the dorsal motor nucleus of the vagus DMV and nucleus accumbens NA. Subsequently selected neurons of the DMV/NA activate post-ganglionic neurons PGN, which result in multiple control actions within the heart.

5. CONCLUSIONS

In summary:

- Vagal outputs and the inputs influencing them are gated into an array of distinct control actions
- Transcriptional regulatory networks in central neurons tune a gradient of attractor like states in a dynamic cellular landscape
- The behavior of these multiscale networks in disease predicts novel targets

Thus, our functional genomic findings forced us into a reconsideration of how cardiac homeostasis works. We next will be interested to see how general these approaches and

concepts may be as to: “How the brain works” and “What is the true neural code.”

Acknowledgements

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