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November 9, 2004

Deborah Boyd
Executive Director
First Candle/SIDS Alliance
1314 Bedford Avenue, Suite 210
Baltimore, MD 21208

Dear Ms Boyd

Enclosed is our progress report covering the first year of our grant 'The Ventral Medulla, Sleep, and Breathing', Number SP0030 (12/1/03-11/30/2004). We certainly appreciate the funding from First Candle that has allowed us to pursue new research avenues. Thank you very much. We look forward to our second year of funding

Sincerely,

Robert A. Darnall, MD
Professor of Pediatrics and Physiology
Director, Training Program in Neonatal-Perinatal Medicine

The Ventral Medulla, Sleep, and Breathing

Progress Report: 12/01/03-11/30/04

a. Specific Aims

The specific aim of the project has not changed and remains: **To determine the effects of destroying groups of medullary serotonergic neurons in the regions of the brain analogous to those found to abnormal in SIDS infants on behavior and circadian sleep and temperature rhythms.**

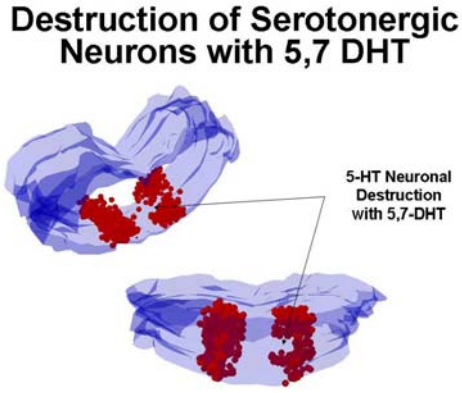
b. Experiments and Results

We have been able to successfully monitor EEG, EOG, neck EMG, ECG, body temperature, and activity in piglets immediately and 1 week after dialysis of 5,7-DHT into medullary groups of serotonergic neurons. 5,7-DHT is a toxin that specifically destroys serotonergic neurons. Since it takes several days for destruction of the neurons, measurements obtained 1 day after dialysis can be used as controls. We have been focusing on the lateral column of serotonergic neurons called the nucleus paragigantocellularis lateralis (PGCL) located in the rostral medulla. Serotonergic neurons in this region project to the spinal cord and other areas of the brainstem, and are thought to be involved in respiratory control and many autonomic functions including thermoregulation, sensory modulation, and sleep. The PGCL is one of the medullary regions found to have abnormal serotonergic receptor binding in a large subset of SIDS infants.

Recently, we have been focusing on heart rate variability as one measure of 'autonomic stability'. We have analyzed various measures of heart rate variability 1 day and 1 week after dialysis of 5,7-DHT into the PGCL. Figure 1 shows the approximate area of serotonergic neuronal destruction. Each red dot represents a tryptophan hydroxylase (TPOH) immunoreactive neuron (i.e. a neuron that synthesizes serotonin). The red dots form the two lateral columns (PGCL). The midline raphé neurons have been taken out of the diagram for clarity. Note the area of neuronal destruction indicated by the arrows.

In this animal we constructed Poincaré plots and calculated changes in spectral power of the inter-beat interval to determine whether destroying a small number of neurons effects autonomic balance. Poincaré plots show the relationship between adjacent R-R intervals (QRS intervals) from the ECG. The shape of these plots are a simple measure of beat-to-beat variability. Broadly taken, the width of the plot in the short dimension is an index of sympathetic/vagal balance.

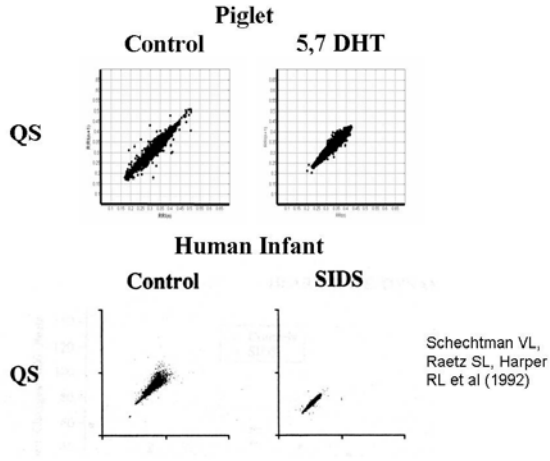
Figure 1



We found in a small group of animals, that the shape of the Poincaré plot was altered after

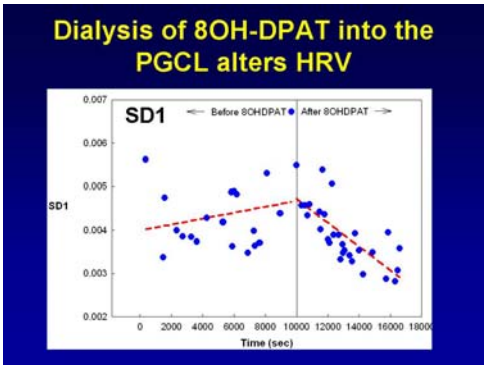
destroying a small number of serotonergic neurons in the PGCL on one side. The upper panels of figure 2 show the Poincaré plots during quiet (NREM) sleep before and after dialysis of 5,7-DHT. Note the narrowing of the transverse dimension of the plot. In the lower panels is an example of Poincaré plots reported by Schechtman and Harper, et al that were obtained from an infant who died of SIDS (right) compared to a plot constructed from a normal control (left). Note the narrowing of the plot obtained from the SIDS infant. Although the similarity of these patterns is not proof of a relationship, it certainly raises interesting questions about the possible roles of medullary serotonergic neurons in modulating autonomic balance. This also raises the possibility that abnormalities in these serotonergic groups of neurons may contribute to increased vulnerability that may not allow the infant to respond to potentially life-threatening stressors often encountered during sleep.

Figure 2



We have obtained similar changes in the width of the Poincaré plot (the statistical measure is termed: SD1) in chronically instrumented piglets after acutely inhibiting the activity of serotonergic neurons in the PGCL. Figure 3 shows changes in SD1 after dialysis of 8OH-DPAT (DPAT) into the PGCL on one side into a chronically instrumented piglet. DPAT decreases the activity of serotonergic neurons by acting on the 5-HT_{1A} autoreceptor located on the soma and dendrites of serotonergic neurons. Each blue dot represents the SD1 derived from Poincaré plot constructed from a single bout of NREM sleep. Note that after dialysis of DPAT, SD1 progressively falls over time.

Figure 3



c. Significance

Many have thought that the etiology of SIDS involves abnormalities in autonomic homeostasis. These studies provide evidence that specific abnormalities of medullary serotonergic neurons may alter sympatho/vagal balance as shown by the alterations in dimensions of the Poincaré plot. This is exciting new information that we will pursue further.

d. Publications and Presentations

The following publications and presentations have credited First Candle during 2004.

Hoffman, JM, Harris, MB, Gill, WH, Niblock, MM, Darnall, RA, Inhibition of serotonergic neurons in the caudal medulla of conscious, awake and sleeping piglets alters the respiratory response to hypoxia, Society for Neuroscience Annual Meeting, San Diego, CA, Oct 27, 2004.

Darnall, RA, Medullary Serotonergic Neurons, Autonomic Homeostasis, and SIDS, The 9th Symposium on Sleep and Breathing, Newport, RI, October, 2004.

Chernov, Mykyta, Analysis of heart rate variability in telemetered piglets before and after destruction of medullary serotonergic neurons. Masters Thesis, Thayer School of Engineering, Dartmouth College (in progress).