Extending the Scientific Legacy of Candace Pert, PhD:
Toward a Model Linking Behavioral, Physiological, & Immune Mechanisms: Implications for Treatment & Prevention

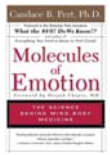
Lydia Temoshok, PhD[1] and Michael R. Ruff, PhD [2,3]
1 University of Maryland, Baltimore, MD, 21201
2 RAPID Pharmaceuticals, AG, Rockville, MD 20850
3 Georgetown University Medical Center, Washington, DC 20007

PNIRS Annual Meeting, May 29, 2014

RAPID PHARMACEUTICALS
Receptor Active Peptides Into Drugs

- ✧ *Neuroscientist*
- ✧ *PNI Pioneer*
- ✧ *Transformative Innovator*
- ✧ *Author*

POPULAR BOOKS



Candace Pert, PhD
Jun 26, 1946 to Sept 12, 2013

Candace Pert died unexpectedly on September 12, 2013, leaving a substantial legacy of scientific discoveries impacting PNI research. She sought to describe a dynamic body-mind informational network by which expression of the emotions, mediated via distributed neuropeptide receptors acting at specific molecular targets in discreet anatomical sites, integrate feelings, behaviors, and immunity to impact health, wellness, and disease. Pert put forth the scientific evidence for these ideas in her seminal paper, "Neuropeptides and their receptors, a psychosomatic network," *(Pert, J Immunol* 1985), and her books *"Molecules of Emotions"* and *"Everything you Need to Know to Feel Go(o)d*. Her ideas were broadly influential and stimulated entire new scientific disciplines, with wide impact that penetrated deeply into popular culture and media.

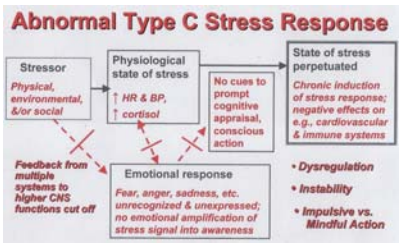
Candace described the **opiate receptor** as a graduate student in 1976, work that won a Lasker prize (for her boss, Solomon Snyder). She then went on to discover other neuropeptide receptors in the immune system. From this foundational work, she was able to identify HIV receptors in brain (1986) and developed the **first HIV entry receptor.** Candace was the first to open a new window into a major peptide signaling system of the brain--chemokines, identified receptors for these peptides, and mapped their neuroanatomical distribution, providing clues to function. She did this two years before the first chemokine, IL-8, was even described, and 10 years before the chemokine receptor for peptide T, CCR5, would be identified (Samson, 1996). Candace was the first to bring a chemokine antagonist into clinical development. Her work identified a path to the highly sought HIV vaccine immunogen, and explained the pathogenesis of neuro-AIDS via chemokine killing of neurons (Pert, 1986; Brenneman, 1988), an effect blocked by Peptide T and its naturally produced homologs, hypothalamic and pituitary VIP/GHRH. She described the **mechanisms of AIDS wasting** in children, and used Peptide T to successfully treat them (Mulroney, 1998; Barbey-Morel, 2002). The NIMH placebo-controlled study of Peptide T and showed **anti-viral benefits** in controlled trials (Goodkin, 2006) and **brain scan benefits** in AIDS (Villemagne, 1996). She solved the problem of peptide aggregation in early trial formulations that were stored for long times, which enabled the resumption of clinical testing. She made her peptide drugs orally active, by methods that are widely generalizable.

Candace viewed the existence of the plethora of shared peptide GPCRs among the brain and body as evidence of the basic integration of body systems to form a dynamic and adaptive psychosomatic network, which has been called *homeodynamic* self-organization (cf. Lloyd, 2001). She believed that the multisystem expression of emotions throughout the brain and body was a path toward such dynamic integration.

Beyond Homeostasis: Toward a Model of *Heterodynamic* Coping

Research on the clinically validated receptor targets described by the action of Peptide T converges on Temoshok's findings from a longitudinal study of 200 mainly African-American HIV+ individuals:* that more adaptive, *heterodynamic* psychosocial and physiological coping with stressors enhance the production of two key β-chemokines which are ligands for CCR5. Production of these chemokines, MIP-1α and MIP-1β, is correlated with more favorable clinical status in persons with HIV (Garzino-Demo et al., 1999), hypothetically because they inhibit HIV entry via the CCR5 HIV co-receptor.

Previously, Temoshok et al. reported on baseline, 24, 36, and 48-month follow-up findings (2008b, 2009a,b, 2010, 2011a,b), which were replicated by the 5+ year results summarized here (also Temoshok, in press a). Using Generalized Estimating Equations to make longitudinal 5+ year predictions, and controlling for age, baseline CD4+ count, medications, and time of measurement, participants showing more chronic emotional dysregulation (higher Type C coping/ Alexithymia scores), and/or more chronic dysregulation of physiological processes (greater heart rate/ blood pressure over-reactivity &/or slower recovery) were significantly more HIV progressed (lower CD4+ cell counts and higher viral loads) at the final 5+ year follow-up. For Type C coping, these outcomes were mediated by higher IL-6 production, which is connected to the inappropriate immune activation central to HIV progression. For the related construct of Alexithymia, as well as for dysregulated physiological responses (HR and BP) to experimental emotional stress scenarios, less favorable outcomes at 5+ years were associated, reciprocally, with the anti-progression factor of lower MIP-1α/ β production.



These findings support the evolution of a model of adaptive coping, as well as its converse: the maladaptive Type C coping pattern (Temoshok 1983, 1990, 1997, 2000, 2002), which has been linked to disease progression in cancer and HIV/AIDS (e.g., Solano et al 2002; Temoshok et al 2008 a,b; Temoshok 2011, 2015a, in press).

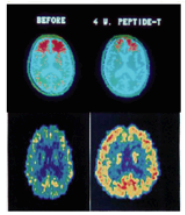
The latest iteration of this model (cf. figures to the left, Temoshok 2015b, in press) highlight why Type C coping is particularly problematic for immune-mediated diseases which may be exacerbated by chronic stress. Like chronic stress, HIV dysregulates the balance between pro- and anti-inflammatory processes, as well as between regulatory T cells and IL-2 producing CD4+ T cells (e.g., Méndez-Lagares et al., 2014).

This latest model of homeodynamic—or what we would call, inspired by Pert's writings, *heterodynamic* coping (reflecting that coping engages multiple systems, via signaling and feedback processes, interacting with changing internal and environmental conditions over time) incorporates mechanisms which could be engaged more efficiently through validated behavioral interventions. Key among these include: behavioral conditioning of immune responses (e.g., Ader et al, 2010), and Mindfulness-based interventions (e.g.,MacCoon et al 2012). Pert's work on identifying viral analogs of presumptive endogenous neuropeptide ligands related to the VIP/PACAP/GHRH peptides suggest additional directions by which behaviors may effect release of peptide hormones from the *endogenous pharmacopeia* of the mind/brain/body.

*Funded by NIH grant R01HD048154, "Biopsychosocial Mediators of HIV progression." (L. Temoshok, P.I.)

Peptide T Blocks Chemokine Receptor: A Treatment & Vaccine for HIV

A. Peptide T treats Neuro-AIDS



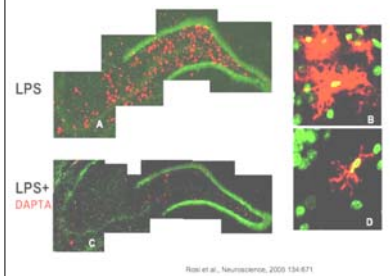
Wetterberg, Lancet, 1986
Low Field MRI
1 wk DAPTA **1 mg bid IV**
3 wks " **2** "

Villemagne, 1995
FDG-PET Study
12 wks DAPTA/ **.4 mg tid IN**

Peptide T plays a major role in the 2013 film The Dallas Buyers' Club

Brain scan improvements after 3 months of Peptide T treatment in AIDS patients

B. Peptide T blocks Inflammation in brain (Rosi, 2005) and in spinal cord in neuropathic pain (Padi, PAIN, 2012)



The figure shows presence of activated microglia in rat brain hippocampus after ICV infusion of the inflammogen LPS. Treating the animals with Peptide T/DAPTA suppressed microglial activation (Rosi et al., Neuroscience, 2005 134:671) and prevents memory loss in APPSweDI/NOS2-/- mice.

C. Creation of a Broadly Neutralizing HIV Vaccine Immunogen (Pert & Ruff, new data, 2014)

Neutralizing antibody IC 50 for affinity anti-DAPTA (HG-1 Rabbit-IgG)

	92RW020	94UG103	92TH021	CMU02	92BR020	JRCSF	NL43	APV13
Clade	A	A	AE	AE	B	B	B	B
HG-1_Rabbit-IgG (ug/ml)	> 0.05	> 0.05	> 0.05	0.02	> 0.05	0.03	0.03	0.02

	APV17	APV6	93IN905	IAVI_C18	IAVI_C22	IAVI_C3	94UG114	aMLV
Clade	B	B	C	C	C	C	D	Lentivirus control
HG-1_Rabbit-IgG (ug/ml)	0.02	0.02	0.02	0.02	> 0.05	0.02	0.02	0.03

Source: Biosciences monogram

➢ Potent Neutralization of 8/15 Clades in the Monogram Biosciences assay. Samples were sent off for third party validation for neutralization potency against global HIV strains from diverse clades with yet another method. A stringent threshold was set. Samples had to have 50% inhibitory activity at doses less than 0.05 ug/ml .

At low doses (.05 ug/ml) a purified Ig blocked many strains, including the fusion hybrid AE example and clades, B,C, and D.

Pert (1986) identified an octapeptide epitope of GP120 which binds to CCR5, and which can be coupled to a carrier protein, like a virus-like particle, to elicit vaccine antibodies, as shown here. Immunogens derived at or near the HIV GP120 V2 peptide T site (e.g., within 10 amino acids of the Peptide T homologous site, residues 188-192 in HXB2) will elicit broadly neutralizing vaccine antibodies against diverse clades (e.g., Ruff, Pert, Pollanova et al., 2007).