NEURONAL CORRELATES FOR THE DEVELOPMENTAL INCREASE OF ANOREXIGENIC DRIVE IN EARLY ONTOGENESIS OF RATS

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The photos show the co-workers of P.G. Kostyuk, Dr. I.V. Melnyk and Dr. N. Pronchuk.

Introduction

In accordance to the modern model of energy balance regulation applicable for the adults, food intake and body weight is regulated by definite hypothalamic nuclei. The primary one is ARC, which senses the blood level of hormones leptin and insulin signaling to the brain about levels of peripheral fat. The activity of arcuate NPY- and pro-opiomelanocortin (POMC) neurons is oppositely modu-

lated by those blood hormones. ARC, in turn, is connected via NPY- and POMC-projections with second-order nuclei creating hypothalamic output: PVN, lateral hypothalamus (LA), and ventromedial nucleus (VMN). It is believed that NPY and α-melanocyte stimulating hormone (α-MSH, cleavage product of POMC) are mediators of natural orexigenic and anorexigenic regulations, as injections of these peptides into the brain stimulate and inhibit food intake, respectively (Elmquist et al. 1999). Biophysically, NPY and α-MSH modulate the activity of second-order neurons acting on their local GABAergic synapses, as has been shown at least for PVN (Cowley et al. 1999). In young postnatal animals, the hypothalamic regulation is apparently different. Thus, ARC does not seem to be functional because it is not yet connected with its projection sites, and local brain injections of leptin and α-MSH do not block the food intake, as in adults. Relevantly, NPY injections are functional already from 2nd PD, which suggests that, primarily, anorexigenic rather than orexigenic drive is highly undeveloped in young mammals. While this obviously has adaptive sense promoting their high metabolism, the neural mechanisms for the increase of anorexigenic drive in early development are not yet known.

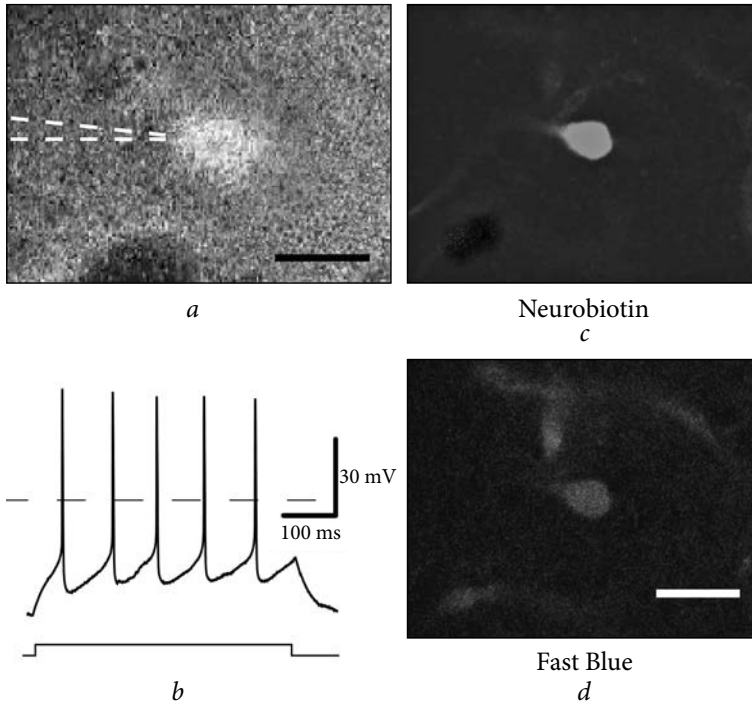

Fig. 1. Identification of neurosecretory parvocellular PVN neurons: *a* — Appearance of a Fast Blue-filled NS cell during experiment. *b* — Recording of a typical firing response. *c* — Post hoc immunostaining for neurobiotin and Fast Blue

Identification of feeding related second-order neurons in PVN

This was done physiologically during patch-clamp recordings in hypothalamic slices from parvocellular neurons, which consist of neurosecretory (NS) and pre-autonomic (PA) subpopulations. NS cells were directly identified by i.p. injections of fluorescent tracer Fast Blue (Fig. 1), captured by axonal terminations of NS cells from blood capillaries in the median eminence and transported back to the soma

PA neurons were identified by their distinctive physiological properties (Melnick et al. 2007). Magnocellular PVN neurons were largely omitted from the study, because they synthesize oxytocin and vasopressin and apparently are involved in the regulation of water but not energy, balance. About 90% of NS and

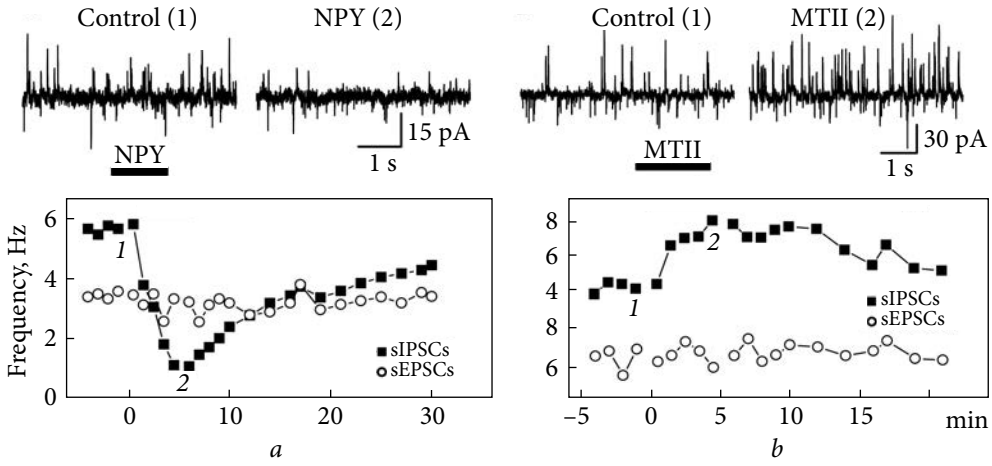

Fig. 2. Depression (*a*) and potentiation (*b*) of frequency of spontaneous IPSCs in NS neuron by NPY and MTII (agonist of melanocortin receptors). 100 nM both

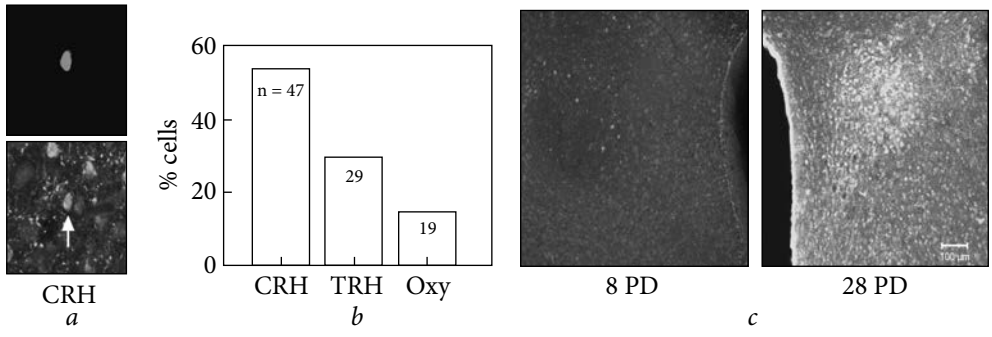

Fig. 3. Peptidergic phenotype of feeding-related neurons, large part of which expressed CRH (*a-b*, red — neurobiotin); *c* — CRH immunostaining of the PVN at 8 and 28 postnatal days, low magnification

40% of PA cells in adult rats are oppositely sensitive to feeding-related peptides, NPY and α-MSH, and therefore are likely involved in regulation of food intake. Biophysically, orexigen NPY decreased, while anorexigen α-MSH increased the frequency of spontaneous GABAergic IPSCs (outwardly directed currents at V_{hold} –40 mV, which was positive for E_{Cl} in our experiments); herein glutamatergic EPSCs (inward) were not affected (Fig. 2).

The neuropeptide phenotype of such neurons was partially characterized by immunostaining for corticotropin-(CRH), thyrotropin-releasing hormones (TRH) and oxytocin (OXY): large part of the neurons contained CRH (Fig. 3, *a-b*), which is known as an anorexigen if injected into the brain. Relevantly, the number of CRH-positive cells and intensity of staining, which indicates the level of the protein expression, increased significantly by 4 PW (Fig. 3, *c*).

Developmental changes of the properties of feeding-related NS cells

Local GABA synapses are the principal target of NPY and αMSH. Thus, the development of these properties was traced. The frequency of both spontaneous (and miniatures, not shown) IPSCs and EPSCs increased by ~6 times between 1st anf 4th PW (Fig. 4). We also checked if physiological data corresponded to the morphologically determined increase of GABAergic synapses. The latter was studied by immunostaining for vesicular GABA transporter (VGAT) (Fig. 5, red), while recorded neurons were injected and stained for neurobiotin (green). The number of presumed synapses (*arrows*) in the dendrites of NS cells increased by ~3.5 times (Melnick et al., 2007).

Finally, the sensitivity of the cells (i.e., of their GABAergic synapses) to NPY and αMSH was studied at different ages. The amplitude of modulatory action

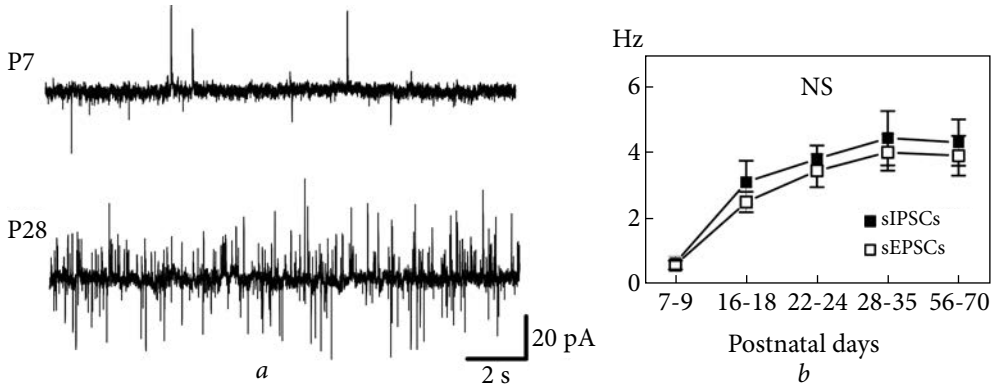

Fig. 4. Increase of frequencies (but not amplitude) of spontaneous synaptic currents with postnatal age

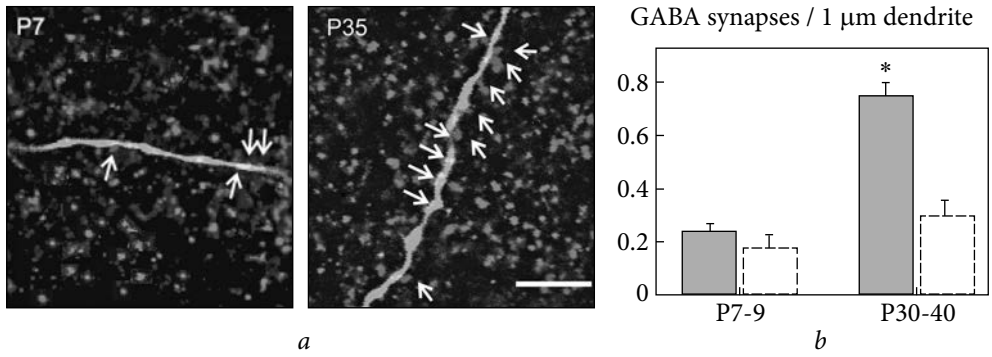

Fig. 5. Developmental increase in the number of GABA synapses (red dots) impinging on the dendrites (green) of feeding-related NS cells, postnatal days 7 and 35 (*a*). Dotted lines indicate statistical expectancy of synapses, if they were randomly distributed (*b*)

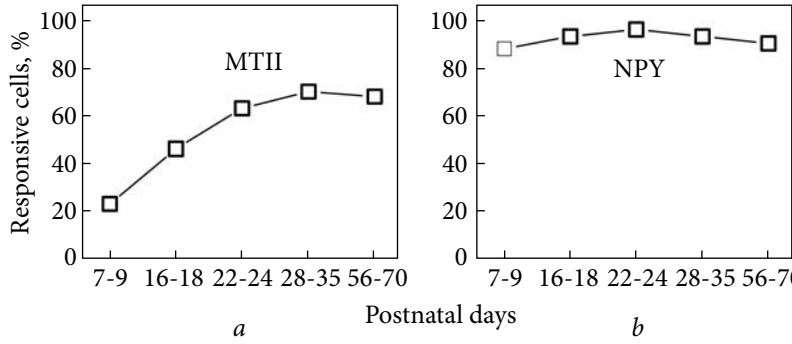

Fig. 6. Developmental increase in sensitivity of feeding-related NS cells to anorexigen αMSH (agonist MTII, left) and no change in sensitivity to NPY (right)

of the neuropeptides was not statistically different, but the number of NS (but not PA) neurons sensitive to αMSH increased critically between 1st and 4 th PW (Fig. 6). In contrast, the sensitivity to NPY was high and constant.

Thus, the reported data, namely strong increase in the number of αMSH-sensitive GABAergic synapses impinging on NS PVN neurons and increased expression of CRH by those neurons possibly constitute the neural mechanisms *on* decreased appetite in early ontogenesis of rats. The data also explain the inefficiency of leptin signaling in early postnatal life.

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