

# The biological basis and clinical significance of hormonal imprinting, an epigenetic process

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**Abstract** The biological phenomenon, hormonal imprinting, was named and defined by us (Biol Rev, 1980, 55, 47–63) 30 years ago, after many experimental works and observations. Later, similar phenomena were also named to epigenetic imprinting or metabolic imprinting. In the case of hormonal imprinting, the first encounter between a hormone and its developing target cell receptor—usually at the perinatal period—determines the normal receptor-hormone connection for life. However, in this period, molecules similar to the target hormone (members of the same hormone family, synthetic drugs, environmental pollutants, etc), which are also able to bind to the receptor, provoke faulty imprinting also with lifelong—receptorial, behavioral, etc.,—consequences. Faulty hormonal imprinting could also be provoked later in life in continuously dividing cells and in the brain. Faulty hormonal imprinting is a disturbance of gene methylation pattern, which is epigenetically inherited to the further generations (transgenerational imprinting). The absence of the normal or the presence of false hormonal imprinting predispose to or manifested in different diseases (e.g., malignant tumors, metabolic syndrome) long after the time of imprinting or in the progenies.

## Introduction

Hormonal imprinting is a basic biological phenomenon which was first observed, named, and defined by us more than 30 years ago. Today, it develops thousands of web-

pages in Google Scholar and hundred thousands of webpages in Google. The phenomenon means that in the developmentally critical periods, animals or their cells memorize normally or pathologically the first encounter with a given hormone or related structures, and this determines the receptors' later binding capacity as well as the reaction of the imprinted cell to the hormone for life (Csaba 1980, 1981, 1984, 2000, 2008). This memory is transmitted to the progeny generations of the imprinted cell. Imprinting with the normal (physiological) hormone is needed for the normal maturation of the receptor (Csaba and Nagy 1985); however, the faulty imprinting as well as the absence of the imprinting can be manifested in diseases or inclination to diseases.

## The phylogenetic basis of hormonal imprinting

The unicellular *Tetrahymena* has binding sites (receptors) for hormones of higher vertebrates (Csaba and Lantos 1973). This observation called our attention to the hormonal system at unicellular level and led to many experiments demonstrating mammalian hormones in *Tetrahymena* and to the successful study of their receptors and transduction pathways (Christopher and Sundermann 1995; Christensen et al. 2003; LeRoith et al. 1980, 1982; Lenard 1992; Kőhidai et al. 2001, 2003; Csaba 2008). These latter were very similar to that of the mammalian ones (Kőhidai et al. 1992; Kovács and Csaba 1997).

*Tetrahymena* memorizes the first encounter with the hormone, and a different (in general more intensive) reaction can be observed in case of the second (and further) encounters. We named the phenomenon to hormonal imprinting (Csaba 1980). The individual life of *Tetrahymena* is very short (few hours only); however, the memory is

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inherited to the progenies and can be observed after hundreds of generations (Csaba 1985, 2008). Imprinting can be provoked not only by hormones but by other molecules which are able to act at receptorial level (Csaba 2008). This seems to be very important for the unicellular population as the cells are able to recognize better and earlier molecules which are dangerous for them and can escape in time. In addition, they are able to recognize more easily useful molecules (e.g., food) far from their sites and can approach them for engulfing. Nevertheless, the phenomenon is important also from evolutionary aspect, as it helps to select molecules suitable for being hormones in the further steps of evolution (Csaba 2008).

### The perinatal hormonal imprinting

Evolution erases the unnecessary mechanisms, while keeping those which are useful and suitable. This suggested the idea that hormonal imprinting, observed in *Tetrahymena* must be present in higher-ranked animals, and the phenomenon must be accomplished in the critical stage of development (Csaba 1984). When—in the first experiment, 34 years ago—newborn rats were treated with high dose of thyrotropic (TSH) or gonadotropic (GTH) hormone, their thyroxine content in the blood of adults was 40–70% less than that of the controls, as a result of faulty imprinting (Csaba and Nagy 1976). Since then, many experiments were done by us and other researchers justifying the need of normal imprinting and the deteriorating effect of faulty imprinting caused by related molecules which are able to bind to the developing receptor (Csaba 1994, 2008; Tchernitchin et al. 1999). However, the target hormone is also able to provoke faulty imprinting, if the amount of hormone or the time of intervention is not suitable (Csaba 1994, 2008; Csaba and Nagy 1976).

Not only polypeptide (as were TSH and GTH and related molecules) or amino acid type hormones acting on cell membrane receptors can provoke faulty imprinting but hormones and hormone-like molecules acting on intracellular (nuclear) receptors as well. These are mainly steroids and the hormones of thyroid gland, T3 and T4. There are many variations in the steroid structure which can bind to the members of the steroid receptor superfamily (Neubert 2002), and our environment is full of synthetic steroids used in medical therapy, or are they environmental pollutants and endocrine disruptors. These can enter into the human organism causing faulty imprinting. It is known that diethylstilbestrol (DES) which was used expansively for protecting endangered pregnancies was bound by the estrogen receptor. In the offspring of such mothers, the vaginal cancer or precancerosis was very frequent (Folkman 1971; Miller 1971). In rats, single perinatal DES treatment decreased the

binding capacity of estrogen receptors to about 60% in adult age (Csaba et al. 1986). Human DES treatment have been forbidden and instead of it, allylestrenol was used. Its human receptorial effect is not elaborated till now; however, in rats, it decreased with 40% the estrogen receptors' binding capacity and destroyed glucocorticoid receptors (Inczeffi-Gonda et al. 1986). Perinatally employed mifepristone or tamoxifen gender dependently influenced the binding capacity of estrogen and glucocorticoid receptors (Csaba and Inczeffi-Gonda 2000). As a consequence of the perinatal treatment, tamoxifen abolished the adult males' and females' sexual activity, while mifepristone stimulated that in males (Csaba and Karabélyos 2001). Single neonatal treatment with dexamethasone decreased dexamethasone binding in adults and increased the receptors' affinity (Inczeffi-Gonda and Csaba 1985). The perinatal treatment with cardioglycosides, digoxin, or ouabain changed the binding capacity of their receptors and also changed cardiac function in adult age (Csaba et al. 1983) as well as neonatal treatment with steroids (Inczeffi-Gonda et al. 1987). Vitamins A and D, months after the perinatal exposure, decreased or abolished males' and females' libido (Mirzahosseini et al. 1996; Csaba and Gaál 1997). The perinatal exposure to environmental pollutants, benzopyrene (present in the exhaust gas of cars), and TCDD=dioxin (which is present in the exhaust gas of diesel motors and cigarette smoke, as well as refuse burners) decrease the number of glucocorticoid receptors and increase the activity of liver enzymes in adult rats (Csaba and Inczeffi-Gonda 1984; Csaba et al. 1991a). Numerous plant-protecting chemicals can bind to the steroid receptors, as vinclozolin, having androgene character and bisphenol, having estrogenic character (Ho et al. 2006; Newbold et al. 2007). Nonsteroidal components present in the soy bean (which has a very important role in our nutrition, especially in the artificial feeding of infants [baby foods]), such as genistein, bind steroid receptors (Martin et al. 1978; Miksicek 1995), and also imprints (Csaba and Inczeffi-Gonda 2002; Csaba and Karabélyos 2002). Imprinting with these steroids decrease the binding capacity of steroid receptors and influence the immune system in adult age as well as the sexual activity of male and female rats (Casanova et al. 1999; Csaba and Karabélyos 2002; Delclos et al. 2001; Guo et al. 2002). These induce several response to estrogen in various target organs including the uterus (Gaete et al. 2010). In addition, numerous other receptor-level-acting molecules cause faulty imprinting (Tchernitchin et al. 1999).

Without the completeness of these data, it can be established that the hormones or molecules which can bind to the hormone receptors, provoke imprinting in the perinatal period, which resulted in alterations of many physiological processes. Imprinting is needed for the normal development and function of the receptor

(Csaba and Nagy 1985); however, foreign molecules—which can bind to the receptor—present in this critical period, cause faulty imprinting with life-long receptorial, biochemical, morphological, and behavioral consequences (Csaba 1994, 2008), and sometimes genetic changes are also provoked, but this effect is exceptional (Igaz et al. 1995; Nelson et al. 1994).

### The late imprinting

The perinatal imprinting is physiological and obligate. It is taking place with hormones which are present in this critical period. There is a possibility of imprinting also in earlier time, in the developing fetus, caused by foreign molecules; however, characteristic is the perinatal imprinting, just after birth, as in this time maternal hormones do not disturb the process and the mother does not protect the infant. In rat experiments, the first 24 h are the most critical from this point of view, which gradually decreases with time. While this obligate general imprintability diminishes, a facultative imprintability appears. This means that continuously dividing cells, which are in a developmental phase, can be imprinted in any time of life, as e.g., the cells of bone marrow, lymph nodes, etc. This type of imprinting is also durable (valid for life). Though this imprinting does not enforce the perinatal imprinting, it results in faulty imprinting with life-long consequences. The imprinters can be hormones or synthetic molecules with hormonal character, drugs acting at receptor level (Cicero et al. 1991), and environmental pollutants similar to the perinatal imprinters. These materials change not only the receptors of their own, but related receptors, too. A single, minimal dose endorphin treatment at weaning life-long influences the endorphin and serotonin content of immune cells, cells of some brain regions, the binding capacity of uterine estrogen receptors, and sexual activity (Csaba et al. 2004a, b, c). Treatment with the tricyclic antidepressant mianserin (Csaba et al. 2003c) as well as H1 receptor blocker terfenadine (Csaba et al. 2003b) durably influence the histamine content of white blood cells. Single treatment with vitamin D3 or dexamethasone at puberty significantly influenced the glucocorticoid receptors' binding capacity in the thymus (Csaba and Inczeffi-Gonda 1999a). The most drastic and extensive effect was produced by the aromatic hydrocarbon, benzopyrene, which provokes imprinting very strongly at different ages (Pap and Csaba 1994; Csaba and Karabélyos 1995; Csaba and Inczeffi-Gonda 1999b; Csaba et al. 2004b). The cells are imprinted also during regeneration (Csaba et al. 1989) and not only hormone binding could be influenced, but the microsomal enzyme system (Csaba et al. 1987), similarly to the perinatal treatment (Ishizuka and Yonemoto 2003).

The results demonstrate that the perinatal imprinting is the obligate; however, faulty imprinting can develop at any phase of life in the actually developing cells. This is justified in case of continuously dividing cells. It can be surmised that it is also taking place in such organs or their cells, which are believed stationary.

### Hormonal imprinting of the central nervous system

For a long time, it was believed that the structural development of the central nervous system is finished at birth. However, recent investigations show that stem cells are present in the brain, which are able to build new structures (Mehler 2008). Any conception is accepted; the fact is that the central nervous system is hormonally imprintable (Csaba and Tekes 2005).

Konrad Lorenz was the first who proposed the “imprinting” name for the phenomenon of behavior of neonatal birds, as a reaction to the first object with whom they meet after birth, independent on being their mother or a spotted ball, and this imprinting has a life-long effect (Lorenz 1957). We used the name hormonal imprinting also to a “first encounter” to the meeting of the receptor and its hormone, generalizing the phenomenon of imprinting to all of the developing organs and cells, and we concretized it to molecules acting at receptor level and to developing receptors (Csaba 1980). The imprinting, proposed by Lorenz is the imprinting of the brain, as it is the setting of the sex when estrogens (transformed from androgens) transform the basic female sex to male sexual behavior (Gorski 2002). This process could be distorted by molecules similar to the sexual target hormones, provoking faulty imprinting. This leads to pathological states. The question is, whether—in addition to this basic phenomenon—other hormones or chemicals can provoke faulty imprinting perinatally or later stages of life.

Beta-endorphin is an endogeneous opioid produced by the code of proopiomelanocortin gene in the brain or other (e.g., immune) cells (Blalock 1998; Csaba et al. 2002). Perinatal endorphin imprinting decreases the serotonin content of striatum, and this stimulates the aggressivity of male and sexual activity of female rats and influences the binding capacity of estrogen receptors (Csaba and Tekes 2005; Csaba et al. 2003a) in adults. At the same time, it increases with magnitudes the concentration of another pain killer, nocistatin, in the cerebrospinal fluid (CSF) (Tekes et al. 2004). Considering that during parturition, the intensity of pain and duration of delivery is individual, the variable endorphin production could influence the aggressivity of adults. Imprinting with the pain-stimulating nociceptin or pain killer nocistatin influences the brain biogenic amine levels in adults, region specifically (Tekes et al. 2009c).

In addition, the effects at weaning also could be settled on the perinatal imprinting. As a consequence of the mother's alcohol consumption, the nocistatin level of CSF and blood increases (Tekes et al. 2007a). Other molecules (benzpyrene, vitamins A and D) also can imprint the brain, is manifested in the change of biogenic amine levels of the brain and CSF (Tekes et al. 2007a, b, 2009a, b, c). Perinatal stress—which disturbs the whole endocrine system—also life-long influences the brain biogenic amine and CSF nociceptin level (Tekes et al. 2010).

The data show that the cells of the brain can be imprinted, similar to the dividing cells. The imprinters could be the brain's own products (in a surplus amount or exogenously given) as well as other molecules able to bind to receptors. It is very difficult to tell whether their effect is manifested through stem cells or through the receptors of matured brain cells, although this latter seems to be likely.

### The mechanism of the imprinting

In the time of recognition of hormonal imprinting, its mechanism was unknown. As the materials provoking imprinting were not known as mutagens, the mechanism was very hardly explainable. Later, genomic imprinting became known and after it, the mechanism of epigenetic processes were studied. This was the time of the recognition of epigenetic imprinting and later the metabolic imprinting, which were hormonal imprinting-like phenomena, and this helped to clear also the mechanism of hormonal imprinting.

The molecules mentioned in the previous paragraphs do not cause changes (mutations) in the genes. However, it is known that the manifestation of genes is regulated by epigenetic factors, and there is a bidirectional relationship between the environment and the epigenome (Mohammad and Baylin 2010; Satterlee et al. 2010). These modifications could be done by DNA methylation, histone modification, or nucleosome positioning (Portela and Esteller 2010). Nevertheless, DNA methylation seems to be the most likely as such a factor during imprinting, which is able to influence durably the manifestation of a gene, as just in the critical phase of ontogeny, this pattern is rebuilding and fixes in the developing cells and their progenies. We believe that our experiment, done 20 years ago, was the first which called attention to this possibility, when methylation of cytosine was inhibited by azacytidine, and this heavily influenced the imprinting in *Tetrahymena* (Csaba and Kovács 1990). Since this time, many experiments were done also in mammals, and these supported the methylation theory of imprinting. Neonatal imprinting with DES provokes a hypomethylation (Li et al. 2003a and b), and dioxin (TCDD) also influences the methylation pattern

(Wu et al. 2004), and this is done by estradiol and bisphenol (Prins et al. 2008) as well as by ethanol (Haycock 2009). In animal and human experiments, the change of the perinatal methylation pattern in depression alters the hypothalamic-pituitary-adrenal axis (Oberlander et al. 2008). Early adversity also influences the methylation patterns (Champagne et al. 2006; Champagne 2010) by drastically disturbing the whole endocrine system in the period of imprinting.

Though our knowledge is sketchy, the effect of imprinters through the change of methylation pattern seems to be likely. This change fixes the information of imprinting and transmit it across generations. This means that hormonal imprinting is an epigenetic imprinting which life-long influences the manifestation of genes without change of the genes themselves. It determines the manifestation of the genes for life or changes and inherits it.

### The transgenerational imprinting

If hormonal imprinting takes place neonatally and its effect is measured when adult, this means that it is transmitted from cell to cell not only in *Tetrahymena* but in mammals as well. This is experimentally justified, indeed (Csaba et al. 1991b). However, the effect of perinatal hormonal imprinting is also manifested in the progeny generations. If neonatal rats are treated with a single dose of insulin and are mated when matured, the insulin binding capacity of the F1 progenies' liver changes: increases in females and decreases in males (Csaba et al. 1984). Similar effect was observed when only one parent was treated. However, in the F1 generation, there is a direct contact between the neonatally treated mother and its progeny. In case of neonatal benzpyrene treatment, F2 generation was studied, and here, the binding capacity of glucocorticoid receptors changed (Csaba and Inczeffi-Gonda 1998) and also the libido of the females (Csaba and Karabélyos 1997) decreased. Fertility was reduced in the F2 generation not only by the decrease of libido, but by the decrease of sperm count and motility (Mohamed et al. 2010). This means that the effect of grandparental imprinting was manifested in the grandchildren. In the F1 generation of DES-imprinted mothers, the frequency of tumors increased (Newbold et al. 2000). Imprinting with receptor level acting vitamins A and D influenced the brain biogenic amine levels of F1 progenies (Tekes et al. 2009a, b, c) as well as the hormone production of immune cells (Csaba et al. 2007). Morphine exposure at puberty caused the increase of anxiety and sensitivity to morphine (Byrnes 2005) in the offspring. These transgenerational effects are characteristic to the aromatic hydrocarbons, to drugs, as cocaine, to phytoestrogens, as the components of soy bean, to the mycokide

and pesticide chemicals, as vinclozolin and bisphenol. The methylation affecting effect of vinclozolin is experimentally justified (Stouder and Paolini-Giacobino 2010). Some nutritional factors, as the folates also have transgenerational effects (Campbell and Perkins 1988; Skinner 2010) and neonatal overfeeding caused glucose intolerance in adult mice, which was transmitted transgenerationally (Pentinat et al. 2010).

The results of animal experiments justify the transmission of hormonal imprinting to the progeny generations and similar or different symptoms are manifested in them. The key factors are the alteration of methylation pattern and epigenetic inheritance (Skinner 2008, 2010; Ho and Burggren 2010; Daxinger and Whitelaw 2010).

### Imprinting and transgenerational imprinting in man

Epidemiological studies demonstrated the presence and effects of hormonal (epigenetic, metabolic) imprinting in man. In the perinatal period of life, the mother prenatally directly or postnatally (through the mothermilk) can transmit imprinters, or this is done also by baby foods. These factors life-long influence the functions of cells and organs (Csaba 2008; Skinner 2007, 2010; Skinner et al. 2010; Grün and Blumberg 2009) as cardiovascular diseases, second type diabetes (Ling and Groop 2009), obesity (metabolic syndrome), certain tumorous diseases, asthma, and obstructive pulmonary disease (Schwartz 2010) can be deduced to the faulty imprinting. Toxic effects, which were believed to be caused by mutations, also can be caused by epigenetic influences (Trosko 2010). The alteration of methylation pattern of genes in the critical developmental phases has a role in the faulty imprinting, and methyl donors (methionin and cholin) and co-factors (folic acid, vitamin B12 and piridoxal phosphate) are needed for the process. In the early period of life, the quality and quantity of nutrition can let or succeed the normal program, or it is reprogrammed. In this latter case, the methylation pattern irreversibly changes, and second type diabetes and pathological glucose tolerance (Holmang 2001; Miles et al. 2005; Signorello and Trichopoulos 1998) appears. In addition to the nutrition factors, estrogens, androgens, progestagens, or similar receptor level acting molecules, as endocrine disruptors (plant protecting chemicals, aromatic hydrocarbons), certain medicaments and hormones, as insulin and leptin can provoke faulty imprinting, as it was shown in case of animal experiments. More observations support the interrelation of adult obesity with perinatal encounter of receptors with the above-mentioned materials (Newbold et al. 2009), as imprinting derails mechanisms which are in connection to weight control (Grün and Blumberg 2009).

The DES catastrophe justified that years or decades after maternal treatment with an estrogen receptor binding synthetic molecule provokes benign or malignant tumors (leiomyoma or adenocarcinoma) in the offspring. The disturbance of methylation pattern can be shown as well as in the case of (not DES provoked) prostate, colon, thyroid, gaster, uterine, and cervical carcinoma (Li et al. 2003b). Similar diseases are caused by perinatal exposure to endocrine disruptors.

A special case is represented by oxytocin which is used worldwide for provoking delivery. In the United States, about 80% of parturitions are done by the help of oxytocin. The oxytocin imprinting—similar to the endorphin imprinting in animal experiments—influences the central nervous system (Plothe 2010). The effect of this imprinting is manifested in infant age in the loosening of infant–mother connections and in the screaming children symptom, when the infant calms only in the arms of an adult. Later, hypotonia or hypertonia could appear as well as absence in the control of head movement. At puberty or in adult age aggression, disturbance in twitting and phobias can appear.

Transgenerational imprinting was observed in human beings mainly in case of nutritional factors (Kaati et al. 2002; Pembrey 2002; Pembrey et al. 2006). Epidemiological statistics justified that the starvation of grandfather in the slow growth phase, just before puberty, when the methylation imprinting of the germ cells happens, increased the lifespan of the grandchildren with years. At the same time, the cardiovascular mortality decreased. In contrast, the overeating of grandfather in the same time decreased the grandchildren's life span with years and four times increased their diabetes mortality. Starvation of the father in similar life period also decreased the cardiovascular mortality of children and the overeating of father protected them from diabetes. These effects were more expressed in grandfather–grandson relation than grandmother–granddaughter relation. Considering this, the sex-specificity is not disclosed (Handel and Ramagopalan 2009), what calls attention to the importance of sex chromosomal genes' methylation. Not only the transgenerational effect of nutrition was observed, but it also was found in case of smoking (Pembrey et al. 2006) and in the case of DES mothers, where hypospadias is more frequent in the progenies (Brouwers et al. 2006).

The study of human imprinting is more difficult than it is in animal experiments. This means that—although the above-mentioned data support the presence and importance of hormonal imprinting and transgenerational imprinting in man—new and thorough investigations are needed for mapping it. The mechanism of the most studied nutritional imprinting also is not cleared: there is a possibility of direct effect to the methylation through methyl donors and co-factors; however, it can be done indirectly by hormones or

by starvation stress, which disturbs the whole hormonal system.

## Conclusions

Considering the above-mentioned data and facts, it is obvious that molecules acting at receptor level provokes imprinting in critical phases of ontogenetic development. The most critical period is the perinatal one; however, in any time of life, imprinting can develop in organs and cells which are in a developing state (bone marrow, brain etc). In the developing embryo, the teratogenic effects are gradually decreasing, and in the fetal period, the imprintability is gradually increasing, reaching the top perinatally, when the physiological imprinting is taking place and the possibility of faulty imprinting by exogenous substances appears.

The physiological imprinting, coded by the genetic program is needed for the normal function of cells (receptors), its absence could be as harmful, as the faulty imprinting. However, faulty imprinting durably alters the function of cells, provoking a state, different from the normal one. It is not sure that this state is wrong or harmful, as there is a chance to improve cell function by stimulating it; however, it differs from the function prescribed in the original program. Nevertheless, more likely that the faulty imprinting spoils the original program, as the partial original program is the suitable, which adapts to the whole program of the organism, and this serves the health of the person. The faulty imprinting can develop in the critical periods (of organ or cell) because the developing receptor is not able to differentiate between the self (target) and related foreign molecules, and these later change the methylation pattern. The functional difference provoked by the faulty imprinting finds room in the physiological variants approaching extreme variants; however, it is not able to tolerate loading. Till now, the role of the amount of imprinter, the time of the intervention, or other accompanied factors are not known.

Hormonal imprinting has a role in the rise of some diseases (Waterland and Garza 1999; Csaba 2008; Plagemann 2006)). This could mean the initiation to or induction of the disease. This means that not only genes can be responsible (not considering outer factors) for the rising of or a resistance to a disease, but also epigenetical endowments (faulty imprinting). As the DES treatment of the mother was responsible for the the vaginal cancer of daughters (and other pathological states also in boys), so as the perinatal soy imprinting could be responsible for the relative resistance to mammary and prostate cancers of the Asian population.

Considering the presence of transgenerational imprinting, we have to suppose that the imprinters alter the methylation pattern not only on the genes of the target cells, but they are able to do it on the germ cells. Theoretically, only these cells would be able to transmit the epigenetic information to the offspring. However, there is a possibility of transmission by the maternal milieu (intrauterine or by mother milk). If the germ cells are the transmitters, this is a regular epigenetic inheritance. If the maternal role is likely, this means a special mechanism characteristic to the imprinting.

Hormonal imprinting is a basic biological mechanism, which sets the receptorial system to the natural environment present in the infant already not protected by the maternal organism. However, this mechanism is not prepared to the mass of imprinters which appear in our present chemically contaminated environment. Proportionally to the increasing number of cars increases, the amount of aromatic hydrocarbons (benzopyrene, TCDD) and steroids are increasing in our drinking water; as a consequence of oral anticonception and plant protection, hormone-like molecules are increasing in our foods; as a consequence of plant protection, agriculture, soy bean, and these materials cause faulty imprinting already perinatally. To this ground, the medical intervention is added, with medicaments acting at receptorial level. The physician does not treat untouched people, but (faulty) imprinted ones, and influence them further. In the early phase of development, the medical intervention also artificially imprints the receptors and not only molecules with DES strength, but by such molecules as perinatal vitamin A and D treatment (McGrath 2001), by surfactants as cortisol, by oxytocin and antihistamines. However, longer is the list of imprinter molecules which were not mentioned, as it is not allowed by the volume of the article, or their imprinter effect was not tested till now, although it would be important, as they are in connection with the endocrine system and/or they act at receptor level (Igaz et al. 2000). This flood of imprinting influences the life of people who were the direct victims of imprinting or transgenerationally inherited it (Jirtle and Skinner 2007). This means that with interventions acting at receptor level in the critical phases of life, we influence not only the life of our own, but the life of the progeny generations (Ho and Burggren 2010), epigenetically intervening to the human evolution (Csaba 2007; Anway et al. 2005; Kaiser 2005; Ubeda and Wilkins 2008; Turner 2009).

Imprinting-like interventions in the critical phases of life must be seriously considered, especially if these are done en masse (as it was in case of DES and its successors, the oral contraceptive agents, or oxytocin). These interventions have to be considered as the factors of the anamnesis as basic factors of therapy and as influencing factors of the

human evolution. As our cells develop in a flood of imprinters, we have to know that receptor level-acting medicaments used in the last decades can have other effects and can provoke other reactions than it was observed earlier, and modern medical care professionals should consider these effects and adapt therapeutical procedures to them.

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## References

- Anway MD, Cupp AS, Uzumcu M et al (2005) Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 308:1458–1469
- Blalock JE (1998) Beta-endorphin in immune cells. *Immunol Today* 18:317–319
- Brouwers MM, Feitz WF, Roelofs LA et al (2006) Hypospadias: a transgenerational effect of diethylstilbestrol? *Hum Reprod* 21:666–669
- Byrnes EM (2005) Transgenerational consequences of adolescent morphine exposure in female rats: effects on anxiety-like behaviors and morphine sensitization in adult offspring. *Psychopharmacology* 182:537–544
- Campbell JH, Perkins P (1988) Transgenerational effects of drug and hormonal treatments in mammals: a review of observations and ideas. *Prog Brain Res* 73:535–553
- Casanova M, You L, Gaido KW et al (1999) Developmental effects of dietary phytoestrogens in Spargue–Dawley rats and interactions of genistein and daidzein with rat estrogen receptors alpha and beta in vitro. *Toxicol Sci* 51:236–244
- Champagne FA (2010) Early adversity and developmental outcomes. Interaction between genetics, epigenetics, and social experiences across the life span. *Persp Psych Sci* 11:564–574
- Champagne FA, Weaver IC, Diorio J et al (2006) Maternal care associated with methylation of the estrogen receptor-alpha 1b promoter and estrogen receptor-alpha expression in the medial preoptic area of female offspring. *Endocrinology* 147:2909–2915
- Christensen ST, Guerra CF, Awan A et al (2003) Insulin receptor-like proteins in *Tetrahymena* thermophila ciliary membranes. *Curr Biol* 13:R50–R52
- Christopher GK, Sundermann CH (1995) Isolation and partial characterization of the insulin binding site of *Tetrahymena pyriformis*. *Biochem Biophys Res Commun* 212:515–523
- Cicero TJ, Adams ML, Giordano A et al (1991) Influence of morphine exposure during adolescence on the sexual maturation of male rats and the development of their offspring. *J Pharmacol Exp Ther* 256:1086–1093
- Csaba G (1980) Phylogeny and ontogeny of hormone receptors: the selection theory of receptor formation and hormonal imprinting. *Biol Rev* 55:47–63
- Csaba G (1981) Ontogeny and phylogeny of hormone receptors. Karger, Basel
- Csaba G (1984) The present state in the phylogeny and ontogeny of hormone receptors. *Horm Metab Res* 16:329–335
- Csaba G (1985) The unicellular *Tetrahymena* as a model cell for receptor research. *Int Rev Cytol* 95:327–377
- Csaba G (1994) Phylogeny and ontogeny of hormone receptors: the origin and development of hormone receptors. *Int Rev Cytol* 155:1–48
- Csaba G (2000) Hormonal imprinting: its role during the evolution and development of hormones and receptors. *Cell Biol Int* 24:407–414
- Csaba G (2007) Thoughts on the cultural evolution of man. Developmental imprinting and transgenerational effect. *Riv Biol* 100:461–474
- Csaba G (2008) Hormonal imprinting: phylogeny, ontogeny, diseases and possible role in present day human evolution. *Cell Biochem Funct* 26:1–10
- Csaba G, Gaál A (1997) Effect of perinatal vitamin A or retinoic acid treatment (hormonal imprinting) on the sexual behavior of adult rats. *Hum Exp Toxicol* 16:193–197
- Csaba G, Inczeffi-Gonda Á (1984) Effect of benzo (A) pyrene treatment of neonatal or growing rats on steroid receptor binding capacity in adulthood. *Gen Pharmacol* 15:557–558
- Csaba G, Inczeffi-Gonda Á (1998) Transgenerational effect of a single neonatal benzopyrene treatment on the glucocorticoid receptor of the rat thymus. *Hum Exp Toxicol* 17:88–92
- Csaba G, Inczeffi-Gonda Á (1999a) Effect of vitamin D3 treatment in the neonatal or adolescent age (hormonal imprinting) on the thymic glucocorticoid receptor of the adult male rat. *Horm Res* 51:280–283
- Csaba G, Inczeffi-Gonda Á (1999b) Direct and transgenerational effect of benzopyrene treatment at adolescent age on the uterine estrogen receptor and thymic glucocorticoid receptor of the adult rat. *Acta Physiol Hung* 86:29–36
- Csaba G, Inczeffi-Gonda Á (2000) Effect of neonatal treatment with mifepristone or tamoxifen on the binding capacity of the thymic glucocorticoid or uterine estrogen receptor of adult rats: data on the mechanism of hormonal imprinting. *Life Sci* 67:2531–2537
- Csaba G, Inczeffi-Gonda Á (2002) Effect of a single treatment (imprinting) with genistein or combined treatment with genistein+benzopyrene on the binding capacity of glucocorticoid and estrogen receptors of adult rats. *Hum Exp Toxicol* 21:231–234
- Csaba G, Karabélyos C (1995) Pubertal benzopyrene exposition decreases durably the sexual activity of adult male and female rats. *Horm Metab Res* 27:279–282
- Csaba G, Karabélyos C (1997) Transgenerational effect of a single neonatal benzopyrene treatment (imprinting) on the sexual behavior of adult female rats. *Hum Exp Toxicol* 16:553–556
- Csaba G, Karabélyos C (2001) The effect of a single neonatal treatment (hormonal imprinting) with the antihormone, tamoxifen and mifepristone on the sexual behavior of adult rats. *Pharmacol Res* 43:531–534
- Csaba G, Karabélyos C (2002) Effect of single neonatal treatment with soy bean phytosteroid, genistein on the sexual behaviour of adult rats. *Acta Physiol Hung* 89:463–470
- Csaba G, Kovács P (1990) Impact of 5-azacytidine on insulin binding and insulin induced receptor formation in *Tetrahymena*. *Biochem Biophys Res Commun* 168:709–713
- Csaba G, Lantos T (1973) Effect of hormones on Protozoa. Studies on the phagocytotic effect of histamine, 5-hydroxytryptamine and indoleacetic acid in *Tetrahymena pyriformis*. *Cytobiologie* 7:361–365
- Csaba G, Nagy SU (1976) Plasticity of the hormone receptors and possibility of their deformation in neonatal age. *Experientia* 32:651
- Csaba G, Nagy SU (1985) Influence of neonatal suppression of TSH production (neonatal hyperthyroidism) on response to TSH in adulthood. *J Endocrinol Invest* 8:557–559

- Csaba G, Tekes K (2005) Is the brain hormonally imprintable? *Brain Dev* 27:455–471
- Csaba G, Inczeffi-Gonda Á, Dobozy O et al (1983) Impact of neonatal treatment with cardioactive glycosides (digoxin, ouabain) on receptor binding capacity, blood level and cardiac function in adult rat. Extension of the imprinting theory. *Gen Pharmacol* 14:709–711
- Csaba G, Inczeffi-Gonda Á, Dobozy O (1984) Hereditary transmission to the F1 generation of hormonal imprinting (receptor memory) induced in rats by neonatal exposure to insulin. *Acta Physiol Hung* 63:93–99
- Csaba G, Inczeffi-Gonda Á, Dobozy O (1986) Hormonal imprinting by steroids: a single neonatal treatment with diethylstilbestrol or allylestrenol gives rise to a lasting decrease in the number of rat uterine receptors. *Acta Physiol Hung* 67:207–212
- Csaba G, Sz S, Dobozy O (1987) Hormonal imprinting of the microsomal enzyme system in adults. Microsomal activity change in response to estrogen (DES, AE) treatment during liver regeneration. *Horm Metab Res* 19:493–496
- Csaba G, Inczeffi-Gonda Á, Dobozy O (1989) Hormonal imprinting in adults: insulin exposure during regeneration alters the later binding capacity of the hepatic insulin receptors. *Acta Physiol Hung* 73:461–464
- Csaba G, Mag O, Inczeffi-Gonda Á et al (1991a) Persistent influence of neonatal 2,3,4,8-tetrachlorodibenzo-*p*-dioxin (TCDD) treatment on glucocorticoid receptors and on the microsomal enzyme system. *J Dev Physiol* 15:337–340
- Csaba G, Bohdanezky E, Köhidai L (1991b) An attempt at transmission of hormonal imprinting between foreign cell lines. *Cytobios* 67:77–83
- Csaba G, Kovács P, Pállinger É (2002) Beta-endorphin in granulocytes. *Cell Biol Int* 26:741–743
- Csaba G, Knippel B, Karabélyos C et al (2003a) Effect of neonatal beta-endorphin imprinting on sexual behavior and brain serotonin level in adult rats. *Life Sci* 73:103–114
- Csaba G, Inczeffi-Gonda Á, Kovács P et al (2003b) H1-receptor blocker antihistamine, terfenadine durably influences the glucocorticoid receptor, and lymphocyte histamine content of weanling rats. *Pharmacol Res* 48:241–244
- Csaba G, Kovács P, Pállinger É (2003c) Prolonged effect of the tricyclic antidepressant, mianserin on the serotonin and histamine content of young rats' white blood cells and mast cells. A case of late imprinting. *Pharmacol Res* 48:457–460
- Csaba G, Kovács P, Pállinger É (2004a) Effect of endorphin exposure at weaning on the endorphin and serotonin content of white blood cells and mast cells in adult rat. *Cell Biochem Funct* 22:197–200
- Csaba G, Kovács P, Pállinger É (2004b) Prolonged impact of five imprinters on the serotonin content of white blood cells of weanling rats: outstanding effect of benzpyrene and chlorpheniramine. *Cell Biol Int* 28:217–222
- Csaba G, Knippel B, Karabélyos C et al (2004c) Endorphin excess at weaning durably influences sexual activity, uterine estrogen receptor's binding capacity and brain serotonin level of female rats. *Horm Metab Res* 36:39–43
- Csaba G, Kovács P, Pállinger É (2007) Transgenerational effect of neonatal vitamin A or D treatment (hormonal imprinting) on the hormone content of rat immune cells. *Horm Metab Res* 39:197–201
- Daxinger L, Whitelaw E (2010) Transgenerational epigenetic inheritance: more questions than answers. *Genome Res* 20:623–628
- Delclos KB, Bucci TJ, Lomax LG et al (2001) Effects of dietary genistein exposure during development on male and female CD (Sprague–Dawley) rats. *Reprod Toxicol* 15:647–663
- Folkman J (1971) Transplacental carcinogenesis by stilbestrol. *N Engl J Med* 285:404–405
- Gaete L, Tchermitchin AL, Bustamante R et al (2010) Biological activity of genistein and soy extracts: selective induction of some but not all estrogenic responses in the prepubertal rat uterus. *Bol Latinoam Caribe Plant Med Aromat* 9:302–311
- Gorski RA (2002) Hypothalamic imprinting by gonadal steroid hormones. *Adv Exp Med Biol* 511:57–70
- Grün F, Blumberg B (2009) Endocrine disruptors as obesogens. *Mol Cell Endocrinol* 304:19–29
- Guo TL, White KL, Brown RD (2002) Genistein modulates splenic natural killer cell activity, antibody forming cell response, and phenotypic marker expression in F (0) and F (1) generations of Sprague Dawley rats. *Toxicol Appl Pharmacol* 181:219–227
- Handel AE, Ramagopalan SV (2009) Public health implications of epigenetics. *Genetics* 182:1397–1398
- Haycock PC (2009) Fetal alcohol spectrum disorders: the epigenetic perspective. *Biol Reprod* 81:607–617
- Ho DH, Burggren WW (2010) Epigenetics and transgenerational transfer: a physiological perspective. *J Exp Biol* 213:3–16
- Ho SM, Tang WY, Belmonte de Frausto J et al (2006) Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. *Cancer Res* 66:5624–5632
- Holmang A (2001) Perinatal origin of adult disease. *Scand Cardiovasc J* 35:178–185
- Igaz P, Tóth S, Csaba G (1995) Long-lasting persistence of elevated sister-chromatid exchange frequencies induced by perinatal benzo(a)pyrene treatment in rat bone-marrow cells. *Experientia* 51:612–615
- Igaz P, Falus A, Gláz E, Rác K (2000) Cytokines in diseases of the endocrine system. *Cell Biol Int* 24:663–668
- Inczeffi-Gonda Á, Csaba G (1985) Prolonged influence of a single neonatal steroid (dexamethasone) treatment on thymocytic steroid binding. *Exp Clin Endocrinol* 85:358–360
- Inczeffi-Gonda Á, Csaba G, Dobozy O (1986) Reduced thymic glucocorticoid reception in adult male rats prenatally treated with allylestrenol. *Acta Physiol Hung* 67:27–29
- Inczeffi-Gonda Á, Csaba G, Dobozy O (1987) Effect of a single neonatal treatment with steroid hormone or steroid-like molecules on myocardial quabain binding in the adult rat. *Gen Physiol Biophys* 6:279–283
- Ishizuka M, Yonemoto J (2003) Perinatal exposure to low doses of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin alters sex-dependent expression of hepatic CYP2C11. *J Biochem Mol Toxicol* 17:278–285
- Jirtle RL, Skinner MK (2007) Environmental epigenomics and disease susceptibility. *Nature Rev Gen* 8:253–262
- Kaati G, Bygren LO, Edvinsson S (2002) Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. *Eur J Hum Genet* 10:682–688
- Kaiser J (2005) Endocrine disruptors trigger fertility problems in multiple generations. *Science* 308:1391–1392
- Köhidai L, Barsony J, Roth J et al (1992) Rapid effects of insulin on cyclic GMP location in an intact protozoan. *Experientia* 48:476–481
- Köhidai L, Keresztesi M, Csaba G (2001) Effect of epidermal growth factor (EGF) on *Tetrahymena pyriformis*. *Acta Protozool* 40:221–224
- Köhidai L, Vakkuri O, Keresztesi M et al (2003) Induction of melatonin synthesis in *Tetrahymena pyriformis* by hormonal imprinting—a unicellular “factory” of the indoleamine. *Cell Mol Biol* 49:521–524
- Kovács P, Csaba G (1997) PLA<sub>2</sub> activity in *Tetrahymena pyriformis*. Effects of inhibitors and stimulators. *J Lipid Mediat Cell Signal* 15:233–247
- Lenard J (1992) Mammalian hormones in microbial cells. *Trends Biochem Sci* 17:147–150



- LeRoith D, Schiloach J, Roth J et al (1980) Evolutionary origins of vertebrate hormones: substances to mammalian insulin are native to unicellular eukaryotes. *Proc Natl Acad Sci USA* 77:6184–6186
- LeRoith D, Schiloach J, Berelowitz M et al (1982) Are messenger molecules in microbes the ancestors of vertebrate hormones and tissue factors? *Fed Proc* 42:2602–2607
- Li S, Hursting SD, Davis BJ et al (2003a) Environmental exposure, DNA methylation and gene regulation: lessons from diethylstilbestrol induced cancers. *Ann N Y Acad Sci* 983:161–169
- Li S, Hansman R, Newbold R et al (2003b) Neonatal diethylstilbestrol exposure induces persistent elevation of c-fos expression and hypomethylation in its exon-4 in mouse uterus. *Mol Carcinogen* 38:78–84
- Ling C, Groop L (2009) Epigenetics: a molecular link between environmental factors and type 2 diabetes. *Diabetes* 58:2718–2725
- Lorenz KZ (1957) Comparative study of behavior. In: Schiller CH (ed) *Instinctive behavior. The development of a modern concept*. Methuen, London
- Martin PM, Horwitz KB, Ryan DS et al (1978) Phytoestrogen interaction with estrogen receptors in human breast cancer cells. *Endocrinology* 103:1860–1867
- McGrath J (2001) Does “imprinting” with low prenatal vitamin D contribute to the risk of various adult disorders? *Med Hypotheses* 56:367–371
- Mehler MF (2008) Epigenetics and the nervous system. *Ann Neurol* 64:602–617
- Miksicek RJ (1995) Estrogenic flavonoids: structural requirements for biological activity. *Proc Soc Exp Biol Med* 208:44–50
- Miles HL, Hofman PL, Cutfield WS (2005) Fetal origins of adult disease: a pediatric perspective. *Rev Endocr Metab Dis* 6:261–268
- Miller RW (1971) Transplacental chemical carcinogenesis in man. *J Natl Cancer Inst* 47:1169–1171
- Mirzahassemi S, Karabélyos C, Dobozy O et al (1996) Changes in sexual behavior of adult male and female rats neonatally treated with vitamin D<sub>3</sub>. *Hum Exp Toxicol* 15:573–576
- Mohamed EA, Song WH, Oh SA et al (2010) The transgenerational impact of benzo(a)pyrene on murine male fertility. *Hum Reprod Adv Acc* 1:1–7
- Mohammad HP, Baylin SB (2010) Linking cell signaling and epigenetic machinery. *Nat Biotechnol* 28:1033–1038
- Nelson KG, Sakay Y, Eitzman B et al (1994) Exposure to diethylstilbestrol during a critical developmental period of the mouse reproductive tract leads to persistent induction of two estrogen regulated genes. *Cell Growth Differ* 5:595–606
- Neubert D (2002) Reproductive toxicology: the science today. *Teratog Carcinog Mutagen* 22:159–174
- Newbold RR, Hanson RB, Jefferson WN et al (2000) Proliferative lesions and reproductive tract tumors in male descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis* 21:1355–1363
- Newbold RR, Jefferson WN, Padilla-Banks E (2007) Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive system. *Reprod Toxicol* 24:253–258
- Newbold RR, Padilla-Banks E, Jefferson WE (2009) Environmental estrogens and obesity. *Mol Cell Endocrinol* 304:84–89
- Oberlander TF, Weinberg J, Papsdorf M et al (2008) Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics* 3:97–106
- Pap E, Csaba G (1994) Benzpyrene treatment in adulthood increases the testosterone level in neonatally steroid (allylestrenol)-treated male rats. *Gen Pharmacol* 25:1699–1701
- Pembrey ME (2002) Time to take epigenetic inheritance seriously. *Eur J Hum Genet* 10:669–671
- Pembrey ME, Bygren LO, Kaati G et al (2006) Sex specific, male-line transgenerational responses in humans. *Eur J Hum Genet* 14:159–166
- Pentinat T, Ramon-Krauel M, Cebria J et al (2010) Transgenerational inheritance of glucose intolerance in a mouse model of neonatal overnutrition. *Endocrinology* 151:5617–5623
- Plagemann A (2006) Perinatal nutrition and hormone-dependent programming of food intake. *Horm Res* 65(Suppl 3):83–89
- Plothe C (2010) The perinatal application of oxytocin and its potential influence on the human psyche. *Internat J Prenat Perinat Psych Med Spring* (in press)
- Portela A, Esteller M (2010) Epigenetic modifications and human disease. *Nat Biotechnol* 28:1057–1068
- Prins GS, Tang WY, Belmonte J et al (2008) Perinatal exposure to oestradiol and bisphenol A alters the prostate epigenome and increases susceptibility to carcinogenesis. *Basic Clin Pharmacol Toxicol* 102:134–138
- Satterlee JS, Schübeler D, Ng HH (2010) Tackling the epigenome: challenges and opportunities for collaboration. *Nat Biotechnol* 28:1039–1044
- Schwartz DA (2010) Epigenetics and environmental lung disease. *Proc Am Thorac Soc* 7:123–125
- Signorello LB, Trichopoulos D (1998) Perinatal determinants of adult cardiovascular disease and cancer. *Scand J Soc Med* 26:161–165
- Skinner MK (2007) Epigenetic transgenerational toxicology and germ cell disease. *Int J Androl* 30:393–397
- Skinner MK (2008) What is an epigenetic transgenerational phenotype? F3 or F2? *Reprod Toxicol* 25:2–6
- Skinner MK (2010) Epigenetic transgenerational actions of endocrine disruptors. *Reprod Toxicol* (in press)
- Skinner MK, Manikkam M, Guerrero-Bosagna C (2010) Epigenetic transgenerational actions of environmental factors in disease etiology. *Trends Endocr Metab* 30:1–9
- Stouder C, Paolini-Giacobino A (2010) Transgenerational effects of the endocrine disruptor vinclozolin on the methylation pattern of imprinted genes in the mouse sperm. *Reproduction* 139:373–379
- Tchernitchin AN, Tchernitchin NN, Mena MA et al (1999) Imprinting: perinatal exposures cause the development of diseases during the adult age. *Acta Biol Hung* 50:425–440
- Tekes K, Hantos M, Csaba G (2004) Single neonatal treatment with beta-endorphin (hormonal imprinting) extremely enhances nocistatin level of cerebrospinal fluid in rats. *Life Sci* 74:1993–1997
- Tekes K, Hantos M, Gyenge M et al (2007a) Perinatal alcohol exposure enhances nocistatin levels in adulthood. *Addict Biol* 12:173–175
- Tekes K, Tóthfalusi L, Hantos M et al (2007b) Effect of neonatal benzpyrene imprinting on the brain serotonin content and nocistatin level in adult male rats. *Acta Physiol Hung* 94:183–189
- Tekes K, Gyenge M, Hantos M et al (2009a) Transgenerational hormonal imprinting caused by vitamin A and vitamin D treatment of newborn rats. Alterations in the biogenic amine contents of the adult brain. *Brain Dev* 31:666–670
- Tekes K, Gyenge M, Folyovich A et al (2009b) Influence of neonatal vitamin A or vitamin D treatment on the concentration of biogenic amines and their metabolites in the adult rat brain. *Horm Metab Res* 41:277–280
- Tekes K, Gyenge M, Sótónyi P et al (2009c) Effect of neonatal nociceptin or nocistatin imprinting on the brain concentration of biogenic amines and their metabolites. *Brain Dev* 31:282–287
- Tekes K, Szegi P, Laufer R et al (2010) Effect of perinatal stress on the biogenic amine neurotransmitter level of the adult rat’s brain. *Int J Dev Neurosci* (in press)

- Trosko JE (2010) A paradigm shift is required for the risk assessment of potential human health after exposure to low level exposures. *Int J Toxicol* 29:344–357
- Turner BH (2009) Epigenetic responses to environmental change and their evolutionary implications. *Phil Trans R Soc B* 364:3403–3418
- Ubeda F, Wilkins JF (2008) Imprinted genes and human disease: an evolutionary perspective. *Adv Exp Med Biol* 626:101–115
- Waterland RA, Garza C (1999) Potential mechanisms of metabolic imprinting that lead to chronic disease. *Am J Clin Nutr* 69:179–197
- Wu Q, Ohsako S, Ishimura R et al (2004) Exposure of mouse preimplantation embryos to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters the methylation status of imprinted genes H19 and Igf2. *Biol Reprod* 70:1790–1797