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Patterns are Universal Restrictions in Space-time, A Theory Exemplified by Sexual Neurogenesis

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A theory is proposed which defines pattern and form as the result of a phase-transition from a homogenous and symmetric state of a system to a state with minimum potential energy. This process is characterized by discontinous changes taking place against the background of space-time. First evidence within biological morphogenesis comes from observations of a sudden departure from "intrinsic growth-direction" in the brain of a recent mutant rat.

INTRODUCTION

Most things and objects which we can find in this world seem to have some degree of complexity: atoms are not merely particles, planets and stars are not simple, spherical objects, rivers are not straight and landscapes are not smooth. In fact we can hardly find anything which is simple, homogenous and isotropic. Even if we try to look at it more closely we carried into more details, a property which has been named "*fractal*". This general and very old observation of a complex and highly inhomogenous world may suggest that complexity and deviation from absolute symmetry (in its physical sense) has a very fundamental meaning, hardly standing back against other laws of nature. One can for example assign some degree of potential energy to a particular state of complexity: a liquid requires a considerable amount of thermal energy in order to preserve its homogenous and isotropic state. If this amount of energy is lost, the liquid will start to crystallise and single domains will settle into particular orientations, giving up their almost perfect symmetry. This liquid has then developed into a specific *pattern* with a given complexity and a reduced amount of potential energy. Thus the observable *complexity* (or pattern)

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of an object can be regarded as the result of a *phase-transition* from a homogenous and symmetric state to a state of broken symmetry. As Andrei Linde and David Kirzhinits have pointed out, this is what could have happened during the early days of our universe, when a transition from a very high energy density (and therefore extreme homogenity) to a lower temperature enabled the appearance of complexity (A. Linde, 1985). With this simple analogy of a crystallizing liquid in mind we can approach the point of the present paper. If we also use the term *pattern* instead of *complexity* or *broken symmetry*, we beginn to understand that all matter in our universe will be part of some kind of pattern (except a perfect fluid, a perfect gas, a perfect human, etc. Who has ever met any ?). It is also immediately conceivable that patterns are not established in a permanent and stable manner as long as energetic fluctuations within an overall cooling universe are appearant. The process of phase-transition between symmetrical and non-symmetrical systems may also be called a *morphogenetic process* and it may be assumed that all matter is subjected to this process. Morphogenesis is characterized by a dynamic change or generation of a set of patterns, where each particular pattern must occur with some kind of *stability*, the pattern with least stability occuring at the end of the process. The thermodynamical interpretation of this concept implies a successive, but discontinous change of a system from high energy into stages with local minimum potential energy (as with the example of crystallization). All discrete energetical stages correspond to particular patterns of a system. Thus *instantaneous transitions* of a system within space-time *restrict* the system into an observable set of patterns. The goal of the present paper should be to provide empirical evidence for this principle by a study of a particular kind of morphogenetic process in biology: the *neurogenesis* of brain sexual-dimorphism in two different rat-strains. After demonstration of *cell-distribution analysis* in the hypothalamus of these animals, a possible theoretical formulation of the observed situation is attempted. This approach is made introducing extremum principles (such as minimum potential energy) into the analysis of diffuse point-processes. This idea goes back to the excellent work on pattern synthesis by Ulf Grenander (Grenander, 1976).

SEXUAL NEUROGENESIS

The cellular development of the vertebrate nervous system can be divided into several, mostly succes steps leading to a complex pattern of cellular connections which is typical for the mature brain. There are progressive changes of the early Anlage, such as cell-proliferation and directed migration and regressive events such as cell-process elimination and selective cell-death (Cowan & Fawcett, 1984). The effect of early cell proliferation on the allometric-growth of neuronal matrix cells and its possible consequences on final brain-shape is shown by S. Fujita (Fujita, this issue).

Not denying the importance of early cell-divisions and the obvious role of location and duration of mitotic activity, a great deal of functional capacity of CNS-structures is acquired during the late phase of post-mitotic pattern-formation (McEwen, 1983; C.D. Toran-Allerand, 1984). During this time

nerve cells "learn" their definitive incorporation within a complex functional circuit. Very frequently this process involves the action of "trophic factors", such as *nerve-growth factor* (NGF) for the outgrowth of sympathetic ganglion-cells (Campenot, 1977) and the trophic effect of certain hormones on the development of specific cell-populations responsible for neuronal sex (overview given in DeVries, et al (eds), 1984). In this study we concentrate on the cell-promoting action of gonadal hormones during the perinatal period in rodents. During this time the brain is imprinted to become either masculine or feminine, independent of the genetic sex of the underlaying cell-genom (Gorski, 1984). As a consequence a striking sexual dimorphism in the preoptic area of the rat anterior hypothalamus develops (Gorski, 1978, 1984). We have investigated this area with the help of quantitative image analysis, estimating the number of neurons per unit volume in dependence of location and orientation of test-fields (Galehr, 1984; Bernroider et al, 1985 and Bernroider, 1985). In addition we have included a recent mutant, the homocygeous Brattleboro-rat which is incapable of synthesising the neuropeptide *vasopressin*. Vasopressin is suspected to play an essential role in the development or maintainance of catecholamine innervation which in turn seems to mediate steroid action on hypothalamic circuits (Sladek et al, 1984; Barraclough, 1983).

The estimation of location-dependent cell concentrations:

Details about the experimental procedure of neonatal hormone-applications are given elsewhere (Galehr, 1984). For short, "*neonatal androgenization*" refers to a single androgen injection s.c. on day 1 after birth into females.
It was our intention to measure the number of neurons within complex test-fields relative to some well defined reference-structure. The cellular architecture of the hypothalamus is sufficiently approximated by an "aggregate of isotropic but non-uniformly distributed particles of spherical shape (nuclei only) from a normal size-distribution (Bernroider, 1985).
A key problem for a suitable sampling procedure in our case was the compensation for non-avoidable deviations in section-plane orientation between single individuals.

This problem has been solved using the following correction. Between a unique dorso-rostral position (CA in fig.1) and a unique ventro-caudal position (NSC in fig.1) the mean number of section planes for all animals was determined. Each randomly selected section number is then corrected according to the difference in observed and expected section number (a more detailed description of this procedure is given in the appendix). This kind of *systematic plane normalization* (SPN) makes it possible to relate all counting results to a 3-dim coordinate system within the hypothalamus. Thus it is a prerequisite for coordinate-dependent Stereology of neuronal cell-distributions.

Further details about the quantitative methods employed for this investigation can be found in Galehr, 1984 and Galehr et al, 1986 in press).

(*) In the APPENDIX the suggested SPN-procedure is shown to be a bias-free correction regarding the expected deviation from the average sectional difference.

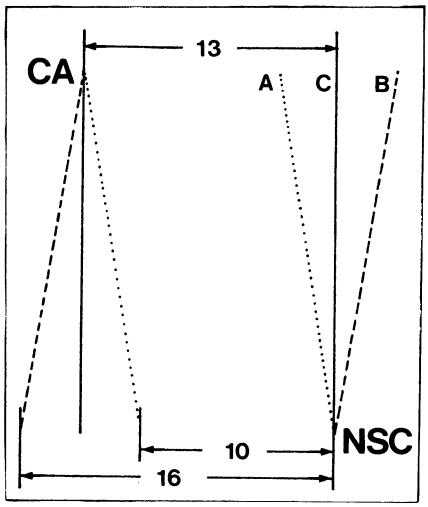

Fig 1: SPN-correction for random deviations in section-plane deviations.

CA: Commissura anterior
NSC: supra-chiasmatic nucleus
(in each case the location is defined as the first frontal section which enables identification).

here: expected deviation = 13
observed (B) = 16
observed (A) = 10

corrected ventral points for (B) e.g. (13-16)=-3 are shifted 3 planes to the right.

Results:

Some of our results are summarized in Fig.2 and Fig.3 below. Both figures show the expected number of neurons relative to their rostro-caudal distribution (means $\pm$ S.E.). A comparison of normal (Spraque-Dawley) males and females (SDM and SDF in fig.2,3) shows two sexually dimorphic zones, where the male clearly exceeds the female in the number of neurons. Brattleboros have a different expression of sexual-dimorphism with a generally increased neuronal density (BRM and BRF). In the present context, the results of neonatal androgenization of females from both strains deserves particular attention: neonatal androgenization in normal females (ASSD in fig.3) increases neuronal density markedly and makes this pattern indistinguishable from its male counterpart. Thus the term *"masculinization"* of female brains may be used. On the contrary, neonatal androgenization of Brattleboro-females (ASBR) reduces neuronal density, draging away from its male-type pattern and becoming indistinguishable from SD-males and SD-masculinized females (indicated by arrows in fig.3). Consequently androgenization in the vasopressin-deficient mutant gives rise to three different patterns (ASBR, BRF and BRM), while normal rats show the expected two types of patterns (SDF and identical SDM-ASSD). This situation is outlined in fig.4. The size of squares in this figure indicates relative neuronal density, dark areas showing *endocrine males* and light areas *endocrine females* ("endocrine" is used to denote "brain sex", independent from genetic sex).

We must conclude that the morphogenetic change observed in ASBR-rats involves a striking departure from an "*intrinsic growth-direction*": while endogeneous androgen during the perinatal period (fig.4 left) pulls an indifferent BR-rat towards its male pattern (masculinization), exogeneous androgen exerts a sudden growth effect in opposite direction.

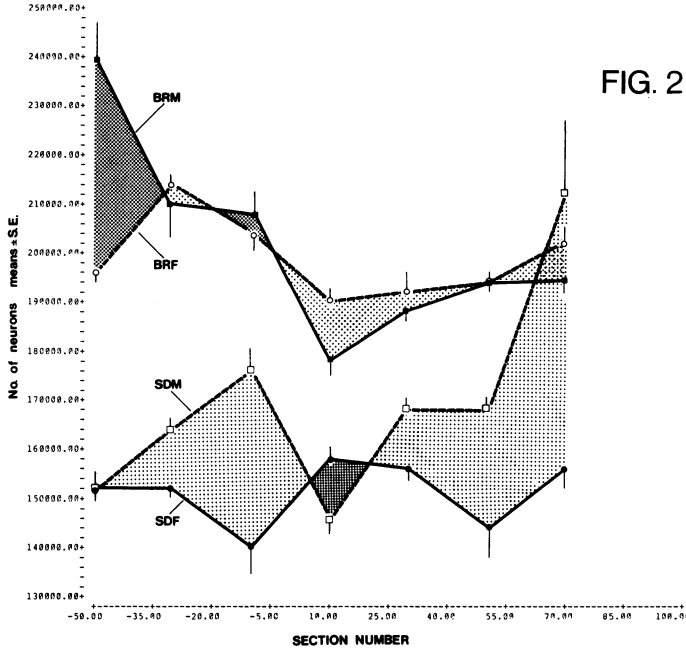
BRM
BRF
SDM
SDF
No. of neurons means ± S.E.
SECTION NUMBER

FIG. 2

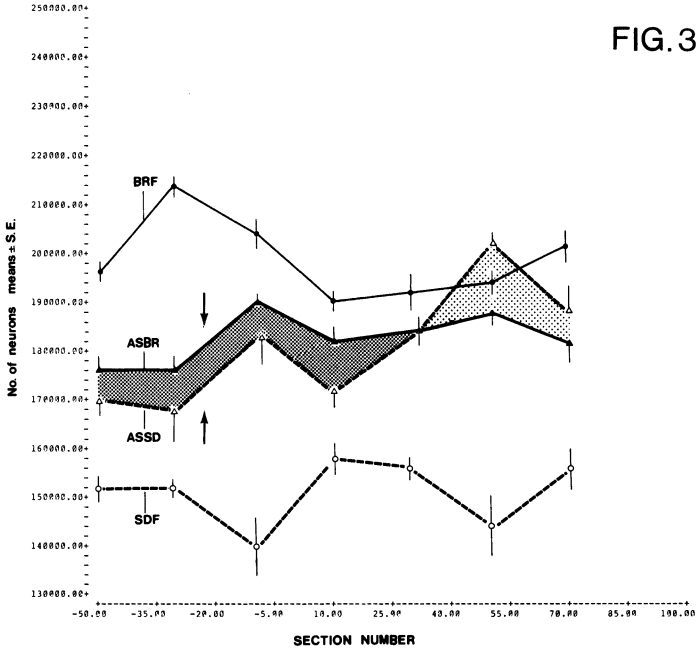
BRF
ASBR
ASSD
SDF
No. of neurons means ± S.E.
SECTION NUMBER

FIG. 3

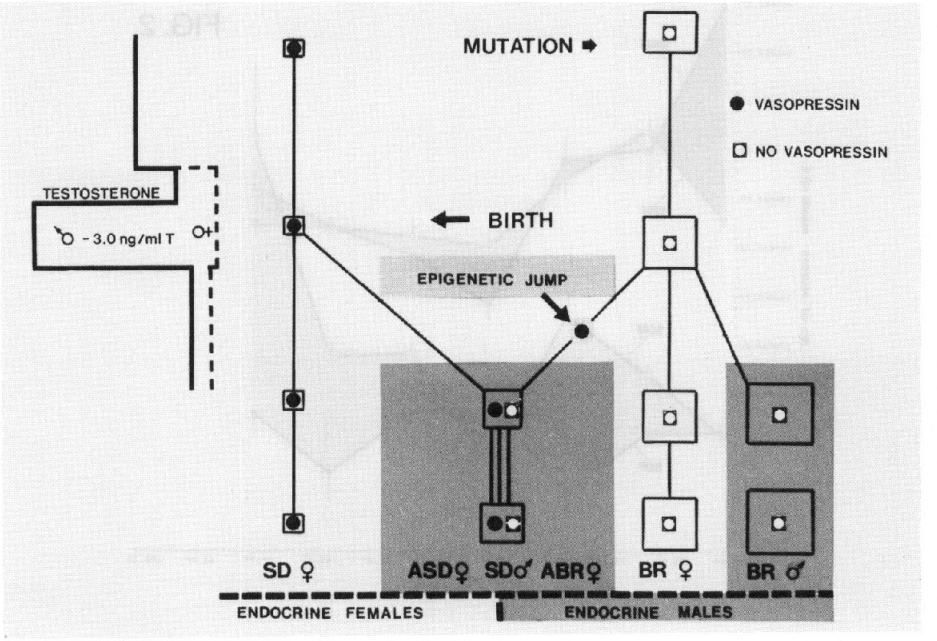

Fig.4

During the perinatal testosterone-peak in males, genetical males develop into endocrine males. Testosterone-application in females (ASD and ABR) results in the same effect in normal SD-rats but in a diverting growth-direction in BR-rats. Note: there are two SD-brains but three BR-brains!

DISCUSSION

The morphogenetic process leading to various neuronal cell patterns as described above shares an important property with the thermodynamic analogy mentioned earlier in this paper. The growth regime under the trophic influence of hormones exhibits two kinds of transitions: - a gradual change of cellular arrangement towards either endocrine female or endocrine male type patterns - and a discontinuous transition from mutant females to normal endocrine males. It is the latter type of sudden transition of a growth pattern which is of particular interest because it displays an instantaneous diversion from an intrinsic growth-direction in genetical females (in figure 4 this transition is called an *"epigenetic jump"*). One could speculate, that the experimental interaction with a prospective ASBR-rat has "destabilized" this pattern and a sudden transition to the "stable" condition of normal androgenized females has occured.

Let us now attempt to model these observations into a more formal shape. In allometric growth a key question arises: if mitosis is not the only way to regulate growth-rate, some additional factor is required to influence growth depending on location. In other words, for epigenetic development the underlaying growth-mechanism will not be "pre-programmed", but be regulated by some external principles. An example for this is *minimum potential energy* for stable patterns in agreement to the stated hypothesis. This entails the introduction of *extremum principles* into the mathematic description of morphogenesis. This idea has been proposed by U. Grenander (Grenander, 1976, 1978).

If P(t) denotes a pattern at time t, I a simple convex image with radius of curvature r, the space-time pattern is determined by a growth-rate f(r) according to

$$\frac{dP(t)}{dt} = I(f(r)) \qquad (1.a)$$

Assuming that growth is accompanied by local energy consumption, depending on two positive constants a,b a possible growth-rate will be

$$\frac{\partial r}{\partial t} = ar - br^2 = br(r_c - r), \text{ with } r_c = \frac{a}{b} \qquad (1.b)$$

If the initial image is roughly but not perfectly circular and $r(\alpha)$ denotes length r in direction α (in $0,2\pi$), the solution of (1.b) shows a change from $r(\alpha,o)$ to r_c as time t increases, according to

$$r(\alpha,t) = r_c \cdot \frac{r(\alpha,o)e^{at}}{r_c - r(\alpha,o) + r(\alpha,o)e^{at}} \qquad (1.c)$$

Thus the final stable pattern will be a circle (r_c), independent of the initial image as long as one does not modify the growth-rate by anisotropic preferences. Without the necessity of such preferences, one could imagine a growth-inhibition effect dependent on either nearest neighbour or long-distance interaction. Both cases are of biological significance. For the present situation nearest neighbour inhibition (e.g. competitive migration) seems to be appropiate. Let i,j denote indices of certain sectors $(\alpha,d\alpha)$ within a (star-shaped) image P, with sector-length $r(\alpha)$ and let c(i-j) be an inhibition constant, depending on the index-difference i-j. The following ordinary differential equation will then simulate a quadratic inhibition between i,j:

$$\frac{dr_i(t)}{dt} = a - br_i(t) - \sum_{j=1}^{n} c_{i-j}\, r_j^2(t) \qquad (2.a)$$

It is extremely interesting to study asymptotic stability for different choices of the parameters a,b,c_i in (2.a). For example U. Grenander has shown, that for r_i=const. or circular shapes for which r_c is given by

$$r_c = -\frac{b}{2c} + \sqrt{\frac{b^2}{4c^2} + \frac{a}{c}} \quad \text{with } c = \sum_i^n c_i \tag{2.b}$$

only circular shapes evolve, but if

$$r = \frac{b}{2c} \pm \sqrt{\frac{a}{c} - \frac{3b^2}{4c^2}} \tag{2.c}$$

which is real and positive for

$$\frac{3}{4} b^2 < a < b^2$$

there can be a sudden change from circular growth-rates to a star-shaped image which is asymptotically stable.

No growth-preferences are preprogrammed here, which is in accordance to the previous assumption that epigenetic growth regimes do not require full genetic information to encode growth direction! The model (2.a) does certainly not simulate geometrical properties of the cell-distributions discussed above, but is capable expressing a key property of the underlaying growth-regime: *instantaneous transitions of a system within space-time with restrictive stability.* A physically more realistic model now requires additional assumptions:

a) the image I is given by the realisation of a diffuse point process in the plane Z = (z_i; i = 1,2,...,n and z = (x,y)).

b) points vanish at time t, $(x,y) \notin I(t)$ with $p_n(x,y)$

c) the history of I(j), j<t affects I(t+1) by either promoting or inhibitory effects.

d) the probability of death in (b) depends on some available energy-flow e(z/l) within a region D_l and a local loss of energy at point z, according to

$e(z) = \prod_i^n f(z-z_i)$ with total absorbance $E = \int_{D_l} e(z)dz$

e) energy flow at z determines either the inhibitory or the promoting effect in (c).

The model we are looking for is then given by the point density $\phi(z)$ for which the functional

$$E = \int_{D_l} e(z/l)\, e(z)dz$$

attains a *minimum* at a time t.
For less complex models and very simple energy flow concepts (e.g. linear gradients along l) some densities $\phi(z)$ under the condition of minimal E have been calculated. More realistic models, taking care of (a)-(e) are presently studied by us.

APPENDIX

Systematic Plane Normalization (SPN):

Let z' be the appearant location of a plane section on the z-axis, further let z_1 and z_2 be the two reference points described above: The difference

$$\Delta z = \mid (z_1-z_2) - E(z_1-z_2) \mid$$

will then be used to correct for plane deviations (where E() denotes the expected difference in section number between z_1 and z_2.

For deviations to the left (Fig.1) $E(z_1-z_2) \leq (z_1-z_2)$ we have

$$(z'+ \Delta z) + E(z_1-z_2) \geq (z_1-z_2)$$

and for deviations to the right (Fig.1) $E(z_1-z_2) \geq (z_1-z_2)$ we have

$$(z'- \Delta z) + E(z_1-z_2) \leq (z_1-z_2)$$

Thus the only points for which no correction is necessary, with $E(z_1-z_2) = (z_1-z_2)$ are those for which $\Delta z = 0$ (equality above).

We now consider the corrected points $(z' \pm \Delta z)$ and estimate their deviation from the expected number of sections between z_1 and z_2:

The left point is $(z'+ \Delta z)$ and the right point is $\{(z'+\Delta z) + (z_1-z_2) - \Delta z\}$ (in Fig.1 these points are (3,16)). With these points the expectation becomes

$$E\{((z'+ \Delta z) - ((z'+ \Delta z) + (z_1-z_2)-\Delta z)) - (E(z_1-z_2))\}$$

Because Δz was defined as $(z_1-z_2) - E(z_1-z_2)$ we finally obtain

$$= E(z_1-z_2) - E(z_1-z_2) = 0$$

Therefore, for corrected points $(z'+ \Delta z)$ the expected deviation from the average sectional difference is equal to zero, which is just the desired property.

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8-2
Q: You appear to have based many of your data on numerical densities. That would be very sensitive to alterations in volume of the reference space. For e.g. you could observe an increase in numerical density which, if accompanied by an undetected decrease in the volume of the nucleus, could add up to an overall decrease in the total number of neurones! You appear to have the relevant information, from serial sections, to calculate the overall volume of the hypothalamus e.g. by Cavalieri's principle. I wonder if you have considered doing this? (V. Howard)

A: The test fields are totally contained in the reference space and we do not refer our results to a "specific nuclear volume" - neither can one calculate the volume of the hypothalamus because the hypothalamus is not a uniquely defined space- e.g. there are no clear boundaries to the 'epithalamus'.

Q: Does a similar difference in neuronal density occur in other species? (H. J. Gundersen)

A: Yes, there is a quite comprehensive list of infra-human mammals, birds and even lower vertebrates which show brain sexual dimorphism. Recently, Swaab and co-workers have found a difference in the human preoptic hypothalamus.