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Growth patterns in the lateral wall of the mouse telencephalon: III. Studies of the chronologically ordered column hypothesis of isocortical histogenesis

P. H. TODD AND I. H. M. SMART

Anatomy Department, Medical Sciences Institute, The University, Dundee, Scotland, DD1 4HN

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INTRODUCTION

In the first paper of this series (Smart & Smart, 1982) it was confirmed that the neurons of the adult mouse isocortex are born in an inside first-outside last sequence. In their original description of this phenomenon Angevine & Sidman (1962) speculated that the reverse order of formation of cortical layers might also apply to the functional columns of the adult cortex described by Mountcastle (1957) and Hubel & Wiesel (1962). However, superimposed on the inside first-outside last trend to the distribution sequence there is an extensive scatter and interpenetration of cell generations. This tends to suggest that the inside first-outside last ordering of cell generations within the cortex is not strict. However, a strict chronological ordering may exist within individual columns and the observed scatter may arise from adjacent columns developing asynchronously. This latter possibility was investigated by measuring the scatter of first generation neurons in autoradiographs of the adult mouse brain which had been pulse-labelled with tritiated thymidine during the period of isocortical cell production. The observed scatter was then compared with that predicted by a model of a theoretical population of neurons accumulating in chronologically ordered columns, from deep to superficial in the order of birth. The correspondence between the observed and predicted distribution was close. Therefore, in spite of the scatter and interpenetration of cell generations, the chronologically ordered column hypothesis remains valid.

The effect of a wave of differentiation passing through a system generating chronologically ordered columns was also examined by setting up a computer model in which pulse-labelling experiments with tritiated thymidine on hypothetical cell populations could be simulated. The correspondence between observed and predicted distributions was again found to be close. The approach has been of considerable value in pointing out the way for future qualitative and quantitative studies.

MATERIALS, METHODS AND RESULTS

Experimental

The experimental material was derived from the photocollages prepared by Smart & Smart (1982). These provided accurate maps of the location of neurons of different labelling intensities at the coronal level of the interventricular foramen in the anterior forebrain of adult mice which had been pulse-labelled with tritiated thymidine on different days of the period of isocortical neuron production in prenatal life.

The photocollages selected for testing were those made at E14, E15 and E16. The

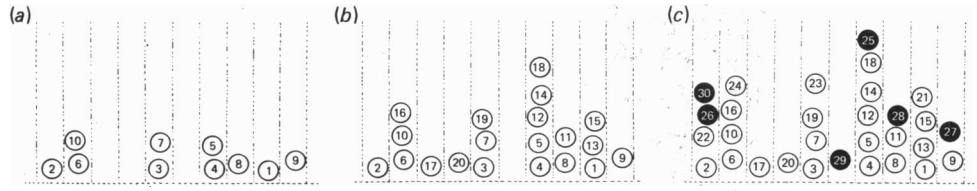

Fig. 1. The figures illustrate successive stages in the envisaged growth of developmental columns. The discs represent neurons entering developmental columns randomly. The inscribed numbers indicate the order in which the neurons were born. The location of the black dics in (*c*) represents the heights achieved by neurons labelled by a pulse of tritiated thymidine present during the S phase preceding the births of neurons 25–30.

E11 and E12 photocollages were discarded because too few cells were present and the E13 because the distribution at this day lay partly in the widely dispersed cells of layer V and partly in the more densely packed layer IV.

A clear acetate sheet was laid over each of the selected collages and, after drawing in the boundaries of the isocortical quadrant, the locations of heavily labelled neurons, i.e. those with more than 25 overlying silver grains per nucleus, were marked. The positions of these nuclei provided a sample of the distribution of the first generation of neurons born after the tritiated thymidine pulse. The acetate sheet was removed and a segment of the middle of the cortical quadrant about one fifth of its circumference was marked off. This portion was judged to be wide enough to contain a sufficient number of labelled nuclei and yet narrow enough not to show any appreciable effect of the ventrodorsal differentiation gradient.

Within the area so demarcated, the shortest distance of each nucleus from the boundary between cortical layers I and II was measured. These distances provided a measure of the height of each nucleus in its postulated column. The results are given in the histograms in Figures 1, 2 and 3 which show the distribution of the depth of labelled cells in the cortex at E14, E15 and E16 respectively.

Theoretical

1. *Derivation of theoretical distribution*

The first task was to derive a theoretical distribution of the heights of neurons labelled at a given stage of development, according to the 'chronologically ordered columns' hypothesis. The hypothesis incorporates the following assumptions:

Associated with each neuron-producing locality there is an array of receptive developmental columns. A newly born neuron is considered to be equally likely to choose any receptive local column and to rise to sit on top of the cells already in the column (Fig. 1). When a column contains a certain number of neurons n, it no longer accepts neurons and ceases to be receptive. A receptive column, thus, has between 0 and n–1 neurons.

It is also assumed that the height of a neuron in the adult cortex is proportional to its height on a developmental column*. If the constant of proportionality between height on the adult cortex and on the developmental columns is c then

(1) the thickness d of the adult cortex and the capacity n of a developmental column are related $d = cn$.

* Here and in the following, the thickness of the cortex is taken to be the perpendicular distance between the boundary of cortical layers I and II and the edge of the corpus callosum; the height of a cell in the cortex is its distance from the corpus callosum, its depth is its distance from the boundary of layers I and II. Obviously, height + depth = cortical thickness.

(2) the tth neuron on a developmental column will appear in the adult cortex at a distance between $c(t-1)$ and ct from the corpus callosum.

Hence, to describe the probability distribution of the height in the adult cortex of a neuron born at a particular stage of cortical development, it is only necessary to know the probability distribution of k, the number of neurons already on the chosen developmental column. Given this, the neuron will sit at position $k+1$ on that column and hence at a height between ck and $c(k+1)$ in the adult cortex.

The autoradiographic experiments under study yield the positions in the adult cortex of populations of synchronously produced neurons. By our hypothesis, the height of a labelled neuron in the adult cortex reflects the number of neurons already on its column at the time of labelling. Because a neuron will not join a full column, the distribution of the height of labelled neurons corresponds to the distribution of the number of neurons on *non-full* columns at the time of injection.

The number of neurons on a non-full column at a given time may be seen to follow a truncated Poisson distribution [Appendix 1]. That is, the probability that the number of neurons on a given non-full column, r, equals k is given by the expression:

$$\Pr[r = k] = \frac{e^{-\theta}\theta^k}{k!} \Big/ \sum_{j=1}^{n-1} \frac{e^{-\theta}\theta^j}{j!} \quad (0 \leqslant k \leqslant n-1). \tag{1}$$

The parameter θ is dependent on the time of injection. We therefore have the probability that the height x of a labelled neuron in the adult cortex lies between ck and $c(k+1)$.

$$\Pr[ck \leqslant x < c(k+1)] = \frac{e^{-\theta}\theta^k}{k!} \Big/ \sum_{j=0}^{n-1} \frac{e^{-\theta}\theta^j}{j!}. \tag{2}$$

Using the Normal approximation to the Poisson, the Probability Density function for x may be seen to be approximately

$$f(x) = \sigma^{-1} Z\left(\frac{x-\xi}{\sigma}\right) \Big/ \Phi\left(\frac{d-\xi}{\sigma}\right), \tag{3}$$

Z and Φ are the unit normal density function and cumulative distribution functions, respectively

i.e. $$Z(x) = \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}x^2},$$

and $$\Phi(x) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{x} e^{-\frac{1}{2}t^2}\, dt,$$

where d is the thickness of the cortex.

$$\xi = c\theta, \quad \sigma = c\sqrt{\theta}. \tag{4}$$

This is the probability density function of a normal distribution truncated at d.

In order to compare the results of the autoradiographic experiments described above with those predicted by (3) it is necessary to assess what stage of development had been reached at the time of labelling in each case; that is to estimate ξ and σ. This may be done from a knowledge of the mean and variance of the heights of observed labelled neurons, using the method of Cohen & Woodward [Appendix 2]. Values of ξ and σ obtained by this method from the material analysed above appear in Table 1.

Using these values the theoretical distributions for the labelled neurons of the experiments were plotted (Fig. 2). These were found to fit satisfactorily the observed data, using a χ^2 goodness of fit test with a 5 % rejection criterion. Values for the χ^2 statistic along with the degrees of freedom may be found in Table 1.

Table 1. *Statistical analysis of depths in adult cortex of cells labelled on different days during the corticogenetic period of prenatal life*

Columns 3 and 4 give the estimates for the parameters $\hat{\xi}$ and $\hat{\sigma}$ of the truncated normal curve. Column 5 gives the estimates for n, the number of neurons in a developmental column. Columns 6 and 7 in the Table perform a χ^2 goodness of fit test between the truncated normal curve and the observed distribution. All measurements in micra.

Day of labelling	d	$\hat{\xi}$	$\hat{\sigma}$	$d\hat{\xi}/\hat{\sigma}$	Degrees of freedom	χ^2
E14	900	613	86	75	7	10·48
E15	900	772	91	83	6	2·85
E16	900	941	100	84·5	2	1·79

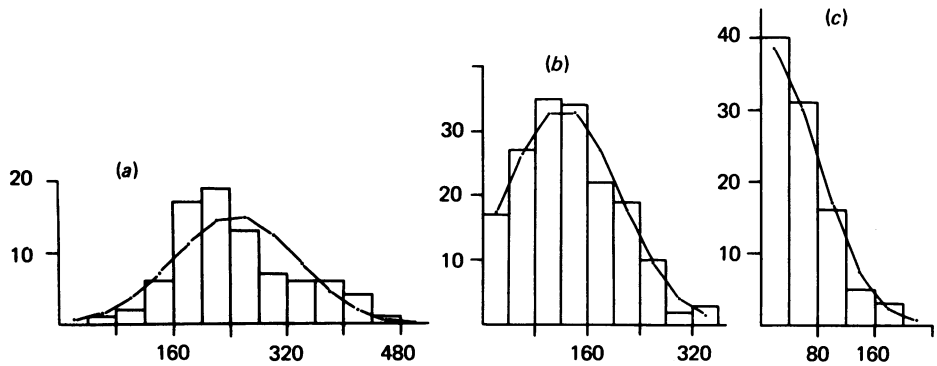

Fig. 2. Histograms showing the spatial distribution of depth in the adult cortex of cells labelled at (*a*) 14, (*b*) 15 and (*c*) 16 days post-conception. Superimposed on each histogram is the curve of the truncated normal distributions with ξ and σ obtained from Table 1. Vertical axis: number of cells. Horizontal axis: depth in cortex (in μm) of labelled cells.

From (4) we see that

$$\xi/\sigma^2 = \frac{1}{c},$$

and hence

$$n = \frac{d}{c} = \frac{d\xi}{\sigma^2}. \tag{5}$$

Hence, the number of neurons in a full column may be estimated by $d\hat{\xi}/\hat{\sigma}^2$, where $\hat{\xi}$ and $\hat{\sigma}$ are the estimates obtained for ξ and σ respectively. This number also appears in Table 1.

2. *A computer model for corticogenesis*

The previous section established that the distributions of labelled neurons predicted by the chronologically ordered column hypothesis corresponds closely with measured distributions. These distributions may be used in a computer model for simulating autoradiographic experiments on hypothetical cell populations.

Here we present a model based on the course of events in the developing mouse cortex. That is, a periventricular proliferative compartment expands under constraints on its thickness imposed by its pseudostratified epithelial structure; differentiation spreads through the system from ventral to dorsal; differentiated neuroblasts

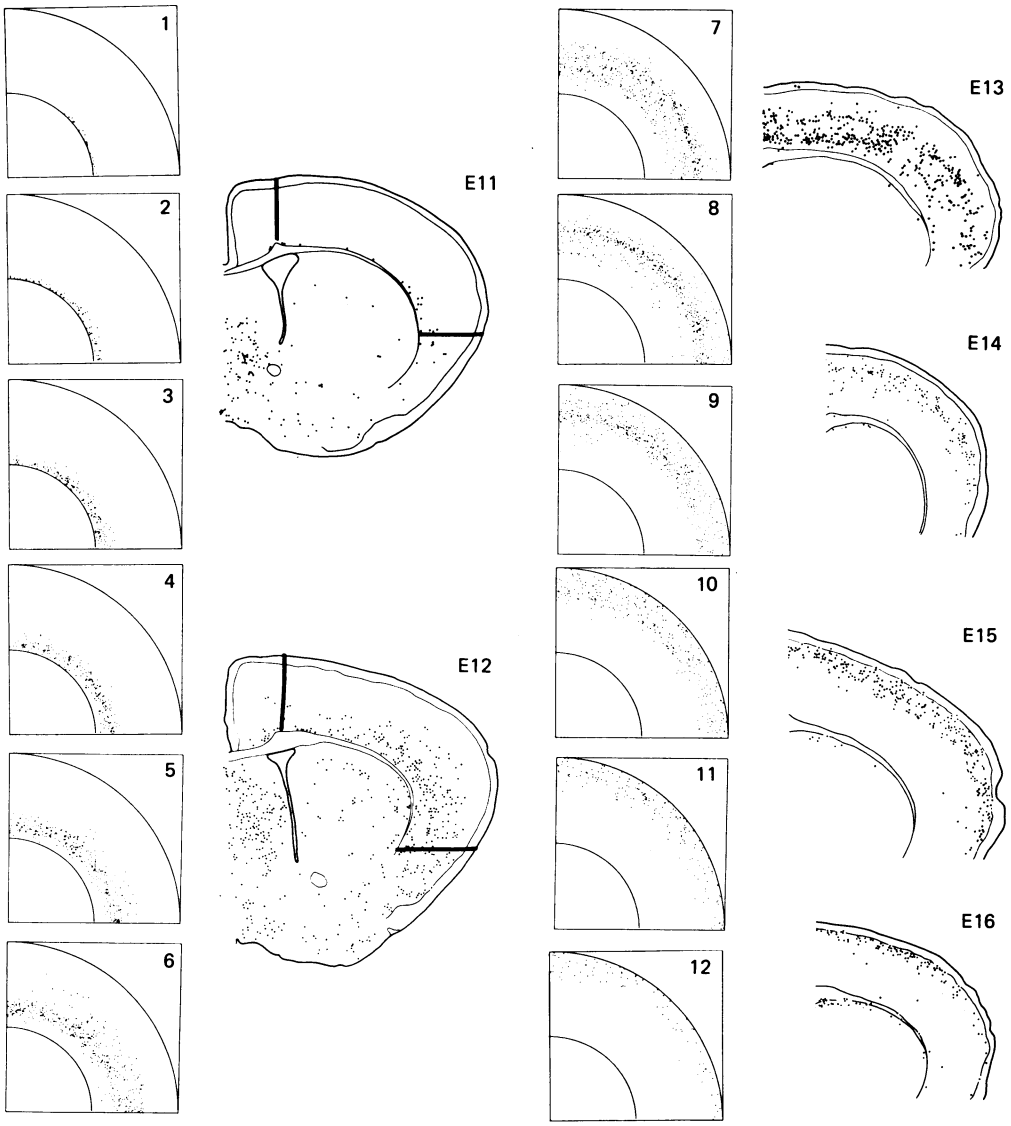

Fig. 3. The quarter-annulus within each square represents the isocortical arc. Within each quarter annulus, distributions of labelled cells predicted by the chronologically ordered column hypothesis are shown after each of twelve successive cell cycles. The number inset in each square indicates the cycle represented. Juxtaposed to the right are the observed distributions of strongly labelled cells in the adult isocortex of mice injected with tritiated thymidine on the stated days between E11 and E16, that is the eleventh and sixteenth days postnatum, taken from Smart & Smart (1982).

migrate radially to form the cortical plate; the cortical plate expands radially to form the cortex.

Notional precursor cells are stored in the computer in a number of finite elements, or compartments equivalent to the radial units discussed in the first two papers of this series (Smart & Smart, 1982; Smart & McSherry, 1982).

Associated with each compartment is a segment of the adult cortex to which it donates neurons: the adult cortex is represented as a quarter-annulus. Precursors

proliferate up to a maximum number per compartment. Precursor proliferation is slowed and eventually reversed by increasing differentiation which is imposed on the system. The differentiation starts in a compartment at one end of the system and travels from compartment to compartment through the system to the other end. To simulate a labelling experiment, the model is run up to the time of labelling, and the number of neurons produced by each compartment is remembered. The number of labelled neurons from each compartment is then calculated and the height of each labelled neuron on its developmental column assessed using the theory developed above. Thus the radial component of each labelled neuron's position in the adult cortex is determined; its angular component is already determined by the compartment which produced it.

The results of simulated labelling experiments carried out at intervals of 1 cell cycle, starting at the commencement of differentiation, are presented in Figure 3. Juxtaposed are the results of real labelling experiments from the first paper (Smart & Smart, 1982), giving the position of strongly labelled nuclei in the isocortical quadrant of adult animals which had been pulse-labelled with tritiated thymidine on successive prenatal days from E11 to E16.

DISCUSSION

Histological studies at the light, scanning and electron microscope level have each demonstrated that during early development the neural epithelium is composed of elongated columnar cells with perikarya at the ventricular surface and peripheral processes extending to the pial surface. These cells are the source of neurons which are released from the ventricular surface and migrate radially to form a separate compartment which is traversed by ventricular cell processes running to the outside surface of the brain. The columnar cells and their processes provide a radial structure to the neuron production and maturation compartments. It is reasonable to regard the accumulations of neurons among the parallel processes as forming columns of cells, each column having been derived from the same part of the precursor layer. The precise histological nature of the developmental columns, and the number of cell types present, are not considered here.

The concept used is of extended columnar cells, not engaged in proliferative activity, providing a columnar structure which is filled in a random manner from an independent proliferative compartment. Although the simplest, the latter is not the only possible realisation of a random element.

We may postulate, for example, that each group of proliferative cells is tied to a specific column. The random element may then lie in deciding which compartment produces a neuron at a given instant, rather than which column a given labelled neuron chooses. With this change in perspective, it may be seen that the theory detailed above still applies. It is not as an attempt to propound one histological view of neuron production that this model is put forward, but rather to suggest that, through some as yet undefined histological process, a regime of chronologically ordered columns is set up during development, whose effects persist into adulthood.

The assumptions on which this theory rests are idealised. Whatever the process, it seems unlikely that the distribution of neurons amongst the columns should be totally random. Rather, the assumption of randomness is an approximation to the effect of a large number of complex factors deciding the destination of a particular neuron.

The assumption of uniformly dense distribution of neurons in the cortex is implicit in the proportional relationship we have assumed between height in the cortex and height in a developmental column. The distribution of neurons is, in fact, not uniform but differs from layer to layer. However, with the exception of layer V, the densities of the layers do not differ greatly and may, as a first approximation, be considered uniform. Distributions where considerable numbers of labelled neurons lay in layer V (i.e. before 14 days post-conception) were not considered.

The estimate for n provided by equation (5) is, nevertheless, based on the assumption of uniform cell density. Hence, variations in the estimate for n are to be expected, due to varying cell densities. The fact that the measurements in this paper were performed in the relatively more dense outer portion of the cortex would tend to overestimate the value of n.

The model for neuron location provided by the chronologically ordered column hypothesis is a simplification of the real system. However, the purpose of any model is to focus on one aspect of a problem by eliminating extraneous detail. The loss in accuracy is made good by a gain in the clarity with which the central aspect of the model is viewed. In this study, we have focused on the chronologically ordered column hypothesis and tested its strength in three ways. Firstly, estimates of n (average number of neurons in a developmental column) were found to be approximately constant through the range of label dates used (Table 1). This indicated that n is, in fact, an internal parameter of the system and suggests that developmental columns have real as well as conceptual significance. Secondly, statistical comparison revealed satisfactory agreement between observed radial distributions of labelled neurons and those predicted by the hypothesis. Thirdly, visual comparison made between maps of labelled cells in the adult cortex and the results of simulated labelling experiments on a population obeying the hypothesis revealed fair agreement between the two distributions.

From these comparisons, it emerges that the chronologically ordered column hypothesis may account for the distribution of labelled neurons in the adult cortex. Although this distribution is doubtless affected by many factors, there is reason for regarding columnar positioning during development as the major factor and others merely as perturbing influences. The strength, then, of the hypothesis is that, with the simplest possible assumptions about cell production and location, it predicts distributions of labelled cells compatible with those observed. It is on this ability to provide a sufficiently close parallel to reality in a sufficiently simplified context that such a model must be judged.

The computer model simulating the effect of a differentiation wave travelling across the proliferative compartment provided corroborative visual evidence in support of the columnar hypothesis. Its main significance, however, is as an example of a model simulating labelling experiments on cell populations whose proliferative behaviour within the restrictions of an epithelial context is known. Such a model may be used in two ways:

(1) To test observed autoradiographic results against some qualitative theory of cell production.

(2) To predict the result of labelling studies on populations whose proliferative characteristics can be quantified.

The model presented in this paper was designed to test the qualitative description of the events of cortical histogenesis of the previous papers of this series. Thus, the results must be interpreted qualitatively and not compared exactly with those of

experiment. It is a subject of further study to develop the second approach; that is, to derive a quantitative model to accept parameters measured from histologica sections (such as rates of cell production, rates of differentiation, etc.) and to predict the quantitative results of autoradiographic experiments. In studies of larger animals, where the expense of radioactive material prohibits comprehensive autoradiography, the most appropriate time to conduct some critical labelling experiment may be determined by using such a computer model.

SUMMARY

The vertical distribution of neurons of different birth dates in the mouse isocortex was measured and compared with the theoretical distributions of neurons accumulating in chronologically ordered columns. The agreement between observed and predicted results was close, so that, in spite of considerable scatter and overlap in the observed distribution of successive generations, the hypothesis that the isocortex is formed of columns of cells arranged from deep to superficial in chronological order of birth is still tenable. A computer model of a differentiation wave travelling across a proliferative system generating chronologically ordered columns was constructed and used to simulate the results of tritiated thymidine labelling experiments. Predicted and observed distributions were again close. The approach has been of value in pointing the way for future qualitative and quantitative studies.

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APPENDIX 1

Given an array of a columns, each with room for n neurons, we seek the probability distribution for the number of neurons on an unfilled column, where the average number of neurons on an unfilled column is m. Each neuron, we have hypothesised, chooses a column at random from the non-filled columns. If b columns are full, then the probability that the neuron picks a particular non-filled column is $1/a-b$. We construct the following equivalent system. Instead of choosing only from the non-full columns, a neuron may choose any column full or non-full. The probability of a particular non-full column being chosen now is $1/a$. If, however, the neuron

picks a full column, then it must choose again. To keep track of this choice we add an extra 'imaginary' neuron to the full column. Full columns, therefore, contain n real neurons plus some number of imaginary neurons, non-full columns contain only real neurons. The probability that a neuron will eventually choose a given non-full column may be seen to be

$$\sum_{r=0}^{\infty} \frac{1}{a}\left(\frac{b}{a}\right)^r = \frac{1}{a-b}.$$

It is clear that, if the non-full columns only are considered, this conceptual system is equivalent to the system under study.

Now the conceptual system yields readily to analysis. When there are N neurons present (including both real and imaginary) the probability that the number of neurons, S, on a given column equals k is

$$\Pr[s = k] = \binom{N}{k}\left(\frac{1}{a}\right)^k\left(1-\frac{1}{a}\right)^{N-k} \tag{A 1.1}$$

This is the Binomial distribution. Because a is assumed to be large, this may be approximated by the Poisson Distribution

$$\Pr[s = k] = \frac{e^{-\theta}\theta^k}{k!}, \tag{A 1.2}$$

where $\theta = N/a$ is the average number of neurons, real and imaginary, per column.

We require the probability that a column contains k neurons, given that it is not full; that is, given that it contains less than n neurons. [Note that if the column has less than n neurons, they must all be real.] By Bayes' Rule

$$\Pr[s = k/\text{column not full}] = \frac{\Pr[\text{column not full}/s = k]\ \Pr[s = k]}{\Pr[\text{column not full}]}$$

$$= \begin{cases} \dfrac{e^{-\theta}\theta^k}{k!}\Big/\sum_{j=0}^{n-1}\dfrac{e^{-\theta}\theta^j}{j!} & \text{for } 0 \leqslant k \leqslant n-1 \\ 0 \quad \text{for } k \geqslant n. \end{cases} \tag{A 1.3}$$

This is a Truncated Poisson Distribution (Johnson & Kotz, 1969). We note that if N_r, the number of real neurons present is known then θ, the average number of real and imaginary neurons per column, must be estimated. An estimator $\hat{\theta}$ for θ may be derived by equating N_r/a with the expected number of real neurons per column from (A 1.2) i.e.

$$\frac{N_r}{a} = \sum_{k=0}^{n} \frac{ke^{-\hat{\theta}}\hat{\theta}^k}{k!} + \sum_{k=n+1}^{\infty} \frac{ne^{-\hat{\theta}}\hat{\theta}^k}{k!},$$

hence

$$\frac{N_r}{a} = n - \sum_{k=0}^{n} (n-k)\frac{e^{-\hat{\theta}}\hat{\theta}^k}{k!}, \tag{A 1.4}$$

and this may be solved numerically for $\hat{\theta}$.

APPENDIX 2

The method of Cohen & Woodward (1953) for deriving $\hat{\xi}$ and $\hat{\sigma}$ estimates for the mean ξ and standard deviation σ of a normal distribution truncated on the right at d is summarised here.

If a sample of size n: $x_1, x_2, \ldots, x_n$ is drawn from such a distribution, then let $\overline{X}$ be the first moment of the sample measurements about the point of truncation.
i.e.

$$\overline{X} = \sum_{j=1}^{n} X_j/n \quad \text{where} \quad X_j = x_j - d.$$

Now writing $\hat{\delta}$ for $\frac{\hat{\xi}-d}{\hat{\sigma}}$

and $Y(x)$ for $1/(Z(x)/(1 - \Phi(X)) - x)$

the following equations yield estimates for $\hat{\xi}$ and $\hat{\sigma}$

$$\hat{\sigma} = -Y(\hat{\delta})\overline{X}, \tag{A 2.1}$$

and

$$\frac{1}{2n}\sum_{j=1}^{n} X_j^2/\overline{X}^2 = \tfrac{1}{2}\, Y(\hat{\delta})\,[Y(\hat{\delta}) - \hat{\delta}]. \tag{A 2.2}$$

Cohen & Woodward (1953) give tables of

$$Y(\delta) \quad \text{and} \quad \tfrac{1}{2}[Y(\delta) - \delta].$$

Inverse interpolation and (A 2.2) suffice to determine $\hat{\delta}$, and the table of $Y(\delta)$ may be used to calculate $\hat{\sigma}$.