The limits of brain determinacy

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The genes do not control everything that happens in a cell or an organism, because thermally induced molecular movements and conformation changes are beyond genetic control. The importance of uncontrolled events has been argued from the differences between isogenic organisms reared in virtually identical environments, but these might alternatively be attributed to subtle, undetected differences in the environment. The present review focuses on the uncontrolled events themselves in the context of the developing brain. These are considered at cellular and circuit levels because even if cellular physiology was perfectly controlled by the genes (which it is not), the interactions between different cells might still be uncoordinated. A further complication is that the brain contains mechanisms that buffer noise and others that amplify it. The final resultant of the battle between these contrary mechanisms is that developmental stochasticity is sufficiently low to make neurobehavioural defects uncommon, but a chance component of neural development remains. Thus, our brains and behaviour are not entirely determined by a combination of genes-plus-environment.

Keywords: Genes; environment; noise; stochasticity; nervous system; brain

1. PHENOTYPIC DIVERSITY IN ISOGENETIC ANIMALS

Even though pure genetic determinism is no longer accepted, it is still widely believed that organisms, including humans, are determined by a combination of genes-plus-environment. For example, in the enormous literature on identical human twins, the differences between them are usually assumed to be due entirely to environmental influences, as is implied by Dalton’s dichotomy ‘nature versus nurture’. The term ‘environmental’ has a very broad scope, referring to any influences coming from outside the body, ranging from prenatal hormonal effects to education and housing.

(a) Intrinsic chance

There is, nevertheless, evidence that genetically identical organisms reared in virtually identical environments differ to some extent, and a few authors have emphasized the need to consider variation owing to chance events that are not specified genetically and seem not to be caused by environmental differences [1–3]. The most extensive treatment of this question is a monograph by Finch & Kirkwood [4], who introduced the term ‘intrinsic chance’ to describe such chance events, and emphasize its long-lasting influence. They support this proposal by pointing out an impressive array of situations in which isogenic animals reared in virtually identical environments show substantial phenotypic variability. Their particular interest is in ageing, and they emphasize the lasting influence of chance events occurring during development. For instance, isogenic nematodes (Caenorhabditis elegans) die over a wide range of ages (10–30 days) even when grown in liquid culture to minimize microenvironmental differences [5]. Finch & Kirkwood attribute this variability to chance events within the organism (although the original authors indicated that still uncontrolled’ environmental effects also played a moderate role).

The introduction of chance into the equation has particular repercussions in the context of the brain, where chance neural events have been attributed a role in psychiatric disease [6], but have also been argued to have positive effects in improved information processing [7], in the avoidance of deadlock in decision-making [8,9], in creativity [10] and in behavioural richness and adaptability [9]. Finch & Kirkwood give some attention to the brain, reviewing subjects such as the variability of neuronal numbers in inbred mice (which are almost identical genetically), and the variability of brain morphology and electroencephalographic activity between identical twins, but there is a need for a more specialized analysis of the role of chance in brain development and function. Another question requiring further discussion is that of whether variations between isogenic animals are truly owing to intrinsic chance events originating within the organism or merely to uncontrolled environmental influences.

The present review addresses these problems by analysing a variety of chance events (noise) that affect many cellular events and intercellular interactions (figure 1), and discusses the importance of the noise in the light of noise-buffering and noise-amplifying mechanisms. The word ‘noise’ has various meanings according to the context, but in this review it always designates events not determined by genes-plus-environment.

For simplicity, I shall ignore the contribution to an organism’s development of non-genetic heritable factors [11] including epigenetic ones [12]. This question is important for the general understanding of heredity, but only marginally relevant to our present concern about the
limits of determinism. I shall also ignore the effects of DNA alterations owing to imperfect mitoses, for lack of space.

(b) Neuroanatomical variability in genetically identical animals

There is no doubt that many of our physical characteristics, such as height and eye colour, are largely determined genetically. Even the brain is to some extent under genetic control, as judged from its gross morphology, but it seems inherently unlikely that so complicated a structure as the brain of higher mammals, containing more than $10^{15}$ synapses in humans, could be completely specified genetically down to the finest detail. Indeed, the brains and behaviour of isogenetic (genetically identical) animals do differ. This is true in mammals, including humans, and in lower animals as well. In monozygotic twins, the differences in brain morphology and behaviour are much less than in dizygotic twins, but they are nevertheless substantial [13]. However, this does not prove a role for intrinsic chance, because many of these differences might be due to environmental factors, which act even prenatally; e.g. one twin may receive a richer blood supply and therefore be better nourished in the womb.

In lower animals, where environmental factors can be better controlled, neuroanatomical differences still occur. For example, in isogenic grasshoppers, there is variability in the positions of neurons and in the branching patterns of their dendrites—as much, in fact, as in heterogenic clutches [14]. Likewise, the axonal branching patterns of identified neurons and their connections vary in isogenic locusts [15]. Similarly, in isogenic daphniae, even though the position, size and branching pattern of each optic neuron is remarkably constant from animal to animal, there is nevertheless some variability in their connectivity [16]. Also, in genetically identical specimens of the tropical fish Poecilia formosa (Amazon molly), it is almost random whether the optic nerves or the Mauthner cell axons cross left over right or right over left [16]. Even among lowly nematode worms, whose development is considered to be much more tightly controlled genetically than that of more complex species, there are differences between the nervous systems of isogenic worms [17].

As the environments of these different isogenic animals were kept as similar as possible, it seems plausible that the variability reflects some kind of intrinsic chance.

(c) Fluctuating asymmetry

A related kind of variability, known as fluctuating asymmetry, is the occurrence of small, apparently random, side-to-side differences between bilaterally paired structures of the same organism. This has been documented in great detail in various organs of many species and constitutes further evidence for the imprecision of genetic control [18]. Fluctuating asymmetry is widely used as a measure of this imprecision (often called developmental instability) and has been shown to correlate with disease and developmental abnormalities; in humans, fluctuating asymmetry in body features (e.g. fingertip pattern) correlates with brain defects [19] and brain-related diseases such as schizophrenia [20,21]. The fact that the side-to-side differences occur within the same organism diminishes the likelihood of their being due to environmental effects, but these can still not be excluded entirely, and the standard view is that fluctuating asymmetry is because of a combination of prenatal environmental stresses and internally generated noise [18]. Determining the relative importance of these two factors is difficult or impossible in mammals, but is in principle possible in lower species through a careful analysis of the effects of varying the environment. Such a study was performed on insect wings; in an aphid clone, where...
the fluctuating asymmetry was particularly high, internal (non-environmental) noise was responsible for as much as 50 per cent of the asymmetry [22].

Thus, the data from between-animal comparisons in isogenetic broods and within-animal studies of fluctuating asymmetry provide a strong case for the incompleteness of determinism by genes-plus-environment. To confirm this indeterminism and understand its origins, it is necessary to analyse the underlying biology.

2. INTRINSIC CHANCE AT THE SUBCELLULAR LEVEL

(a) Thermal movements and incoordination underlie intrinsic chance

It is generally accepted that the genes do not control everything that happens in a cell and that intrinsic chance (as defined above) therefore plays a role. All agree that thermal movements are an important source of intrinsic chance, and they may even be its sole active source, but I shall also use the term incoordination, as described below and shown in the left side of figure 1.

Thermal movements are of two main kinds. First, in the warm, wet milieu of a cell, every free molecule is constantly undergoing thermally induced displacements that underlie diffusion, a process fundamental to cellular physiology. Second, internal vibrations underlie spontaneous conformation changes in large molecular structures such as proteins and protein complexes, and these are particularly important in ion channels and receptors. Thermal movements are not determined by the organism’s genes and/or environment. It is true that an embryo’s temperature depends on environmental effects, but the temperature determines only the magnitude of the thermal movements, and not the details of their timing and direction. This is not to deny that the movements are determined by the laws of physics (apart from Heisenbergian uncertainty, etc.), but they are not determined by genes-plus-environment, so for the present purposes they may be considered stochastic.

I use the term incoordination to refer to the fact that the convergence of independent causal chains is not perfectly orchestrated by genes-plus-environment. In other words, uncontrolled coincidences occur. Incoordination affects both intracellular and intercellular interactions. Within a cell, for example, an enzymatic reaction may depend on both the activation of the enzyme and the provision of its substrate, but the spatio-temporal convergence of the two will involve a chance component. An example of intercellular incoordination is provided by receptor-ligand interactions, for which the meeting of a ligand and its receptor will involve a chance component. Incoordination will be enhanced by thermal movements and the non-directedness of diffusion, but may occur even where directed events are concerned (e.g. active transport along microtubules), because the genes do not prescribe the precise spatio-temporal convergence of independent pathways.

Central to this review is the question of whether these two sources of intrinsic chance, thermal movements and incoordination, have important effects on cellular and organismic phenotype and function. First, I discuss their effects at the subcellular level, and then the consequences at higher levels such as neural circuits.

(b) Gene expression noise

The term ‘gene expression noise’ is generally used in a broad sense referring to the stochastic component of protein synthesis, which reflects the noisiness of both transcription and translation. It occurs because both these processes depend on the random diffusion and jostling and life and death of molecules. When large numbers of molecules are involved, the effects of the randomness will be averaged out by the principle of mass action, but the numbers are not always large. There are often less than 20 mRNA molecules for a given gene [23], and in some cases, the number of transcription factor molecules is also very low [24]. The study of gene expression noise has expanded greatly over the last decade into a flourishing research area, although most of the experimental work has focused on bacteria and yeast. There are some data from cells of higher eukaryotes [25] including humans [26], but there appear to be none from neurons.

The magnitude of gene expression noise varies between genes. Those whose deletion would be lethal to the organism and genes that encode subunits of multi-protein complexes tend to have less variation, suggesting a functional need for noise minimization [27]. On the other hand, highly regulated genes exhibit greater than the average expression noise. This is hardly surprising, as regulation (by transcription factors) is the source of the extrinsic noise component, and it appears to reflect a natural trade-off between the need for a gene’s expression to be regulated, providing a wide dynamic expression range, and the need for its noise to be minimized [28]. Other causes of the trade-off exist, involving chromatin remodelling or regulation at the mRNA level or post-translational mechanisms, and are discussed in the study of Mameli [11].

The importance of gene expression noise for cellular function and phenotype has been studied in yeast and bacteria and in computational models of genetic networks; the noise-induced fluctuations in protein levels are clearly sufficient to have important effects [29,30]. In higher eukaryotes, the same noise phenomena occur, although their consequences have been less well studied. Their analysis is complicated by the existence of an additional source of expression variability owing to chromatin remodelling, which is a regulated phenomenon and not noise (although it may include a stochastic component). It causes genes to make a transition between an active state associated with ‘open’ chromatin, and an inactive one (‘closed’ chromatin). This variability causes a correlation between the temporal expression patterns of genes that are adjacent on the same chromosome, but a lack of correlation between remote genes, or between two copies of the same gene on different chromosomes [29].

(c) Channel noise

Noise in ion channels is another thermally induced phenomenon. The electrical activity of the brain depends largely on voltage changes owing to the flow of ions such as sodium, potassium, chloride and calcium through channels in neuronal membranes. In addition, the calcium ion is an important second messenger, with powerful effects on intracellular signalling pathways. Ion channels are usually composed of a small number of transmembrane protein subunits that combine to form a

very narrow pore through which certain ions can travel in single file. Most channels can be in one of three different states: open, closed or inactivated (closed and incapable of opening). I here focus on the first two.

The channels switch rapidly between the open and closed states when the channel subunit proteins switch between alternative stable conformations. This occurs spontaneously because of thermal agitations, but the probability of switching is influenced importantly by the binding of particular ligands (e.g. neurotransmitter molecules) to receptors adjacent to the channel, or by the voltage across the membrane, or by a combination of the two. The precise moment of switching is not currently predictable, and is certainly not determined by the cells’ genes or external environment, so the switching process is considered stochastic [31,32].

Through its effects on membrane potential, channel noise contributes to synaptic noise (see below) and can have major effects on the timing of action potentials, affecting both their triggering and their propagation. The effect on their time of triggering is greatest when this occurs near threshold, and is greatest in small neurones because the effect of an ion channel is proportional to the membrane resistance. In exciticable cell bodies of less than 3 \( \mu \text{m} \) diameter, or in axons of less than 0.3 \( \mu \text{m} \) diameter, the membrane resistance is sufficiently high for the spontaneous opening of a single \( \text{Na}^+ \) channel to provoke a ‘rogue’ action potential, which imposes a lower size limit on the sizes of neuronal cell bodies and axons [7]. For similar reasons, the speed of action potential propagation is variable in very thin axons because of channel noise at nodes of Ranvier, and simulation indicates that this could cause jitter of about 2 ms in a 0.2 \( \mu \text{m} \) thick cerebellar parallel fibre 1.4 mm long [33].

The importance of channel noise for neuronal development is twofold. First, channel noise affects intracellular signalling in developing neurons via its effects on calcium flux. Second, as electrical activity has major effects on development (see below), the modification of electrical activity by channel noise must be expected to affect neural development.

(d) Synaptic noise
The term ‘synaptic noise’ refers to uncontrolled variability in synaptic transmission, which can be considerable. It is caused by several different uncontrolled events. These include variations in the production of the receptor and channel subunit proteins owing to gene expression noise (§3.2) and channel noise (§3.3) in presynaptic calcium channels and in postsynaptic receptor channels, but also the spontaneous opening of intracellular calcium stores, spontaneous changes in the presynaptic calcium sensor protein (synaptotagmin-1) and random effects in neurotransmitter diffusion [33].

(e) Other sources of noise in cells
Gene expression noise, channel noise and synaptic noise are only examples of the situations where thermal movements and incoordination lead to incompletely controlled cellular events. This is likely to occur in any situation where molecular numbers are low, as is quite common. For example, the number of transmitter receptors per synapse is very variable and often quite low; on hippocampal pyramidal neurons, for commissural or association inputs, the number of AMPA receptors per dendritic spine has been calculated to range from less than 3 to about 140 [34].

Low molecular numbers are also likely to occur in very small cellular compartments such as synaptic vesicles, which are typically 50 nm in diameter (sometimes as low as 15–20 nm). A single 50 nm vesicle contains only about 200 protein molecules in all, of 20–40 varieties, and probably only one or two copies of the proton pump [35]. In small compartments such as these, not only molecular fluctuations but even changes in the numbers of protons and electrons may be important. If the effects of proton buffering are naively ignored, a simple calculation gives the absurd result that 50 nm vesicles at pH 5.5 contain only one free proton per 43 vesicles!

In these cases, however, the effects of the fluctuations will, to some extent, be averaged out because there are several vesicles per synapse—typically 50–100 in the brain [36], although only one, at most, releases its content in response to an action potential—and because there are many synapses per neuron (typically a few thousand in the central nervous system).

3. SUBCELLULAR CHANCE EVENTS AT THE LEVEL OF NEURONAL CIRCUITS

(a) Stochasticity in neurite growth
The growth of neurites (axons and dendrites) is not random; they seek particular targets. But stochasticity is fundamental to the target-seeking, which involves semi-random events and subsequent selection. Many of the principles of axonal growth apply to cell migration and dendritic growth as well, but I shall focus on axons because their guidance mechanisms have been studied in great depth and detail [37,38]. For this very reason it will be necessary to simplify greatly.

The advancing end of the axon (or the dendrite) is called its growth cone. This is a highly active structure from which finger-like actin-filled extensions called filopodia are constantly growing out and often retracting as they test out different possible pathways on a trial-and-error basis. Just as a traveller can respond to global cues such as the direction of the sun or a compass bearing, and to local cues such as ‘turn left after the house with red shutters,’ so do axons. Global cues for filopodia (and hence for growth cones and their axons) include gradients of diffusible molecules and possibly electrical fields. Local cues are provided by a considerable variety of guidance molecules in the membranes of other cells or in the extracellular matrix, which can either attract or repel the filopodium [39,40]. The attraction or repulsion depends on receptors that are expressed at the filopodial tips and activate signalling pathways within the filopodium when it encounters a guidance molecule. Not only do the growth cones respond to the tissue through which they grow, but they express new receptors as they advance through it, so as to be able to respond to new cues. Later, growing axons often follow the ‘pioneer axons’ that have already grown, with the result that axons tend to be fasciculated (bundled) together, but they may need to defasciculate at a particular point. Crucial points where axons need to change direction or leave an axon bundle are called ‘choice points’ [40]; here,
strategically placed groups of cells called guidepost cells, which are often immature neurons, express guidance molecules.

It is generally accepted that the above-described processes include a random element [38]. The initial outgrowth of individual filopodia to sample their environment is thought to be essentially random, the growth being directed by the selective stabilization of filopodia that receive positive signals. Even this stabilization has a stochastic component. At the size-scale of individual filopodial receptors, cues provided by gradients of diffusible molecules are presumably stochastic, as their diffusion to a particular receptor will depend on the thermally induced random motion of the molecules. Likewise, the finding of guidance molecules on cells or extracellular matrix in the axonal path has been likened to a blind woman finding her way by tapping her cane from side to side [38]; the initial sensing is a form of stochastic sampling whose rate needs to be in relation to the axon’s speed of advance. Modelling of the growth cone response to a gradient of trophic factor shows the importance of noise [41].

(b) Axonal targeting errors and their elimination
Despite the multiplicity of the local and global guidance cues, errors in axonal growth occur, confirming the presence of a stochastic component to the growth cone guidance mechanism. This was first suggested by Ramon y Cajal who described what he called ‘strayed’ fibres in the cerebella of various very young mammals [42]. His subsequent studies led him to conclude that the errors were eliminated during development.

The discovery in the 1970s and 1980s of transient neural connections in many neural systems confirmed Cajal’s interpretation, but showed that not all transient connections are aberrant [2]. The well-known phenomenon of ‘exuberant connections’, which involves the transitory formation of massive connections that are then pruned, as in the corpus callosum of young kittens [43], is not considered to represent developmental errors but rather seems to be a deliberate strategy for generating specificity by axonal pruning.

We are here concerned with a different, smaller scale and more variable phenomenon that can more plausibly be attributed to errors in axonal pathfinding. For example, in birds such as chickens and pigeons, there is a strong centrifugal projection to the retina from a nucleus at the junction of the midbrain–hindbrain, the isthmo-optic nucleus (ION). In adults, this projection is entirely contralateral and has a strict topographical organization, but in chick embryos two kinds of axonal targeting ‘errors’ have been identified. Some axons appear to make errors as they grow through the optic chiasm, because initially a small percentage of them (just over 0.1%) project to the ipsilateral retina, not the contralateral. These aberrant axons are subsequently eliminated during the ION’s period of naturally occurring neuronal death, when about 55 per cent of its neurons die (between embryonic days 12 and 17) [44,45]. In this case the neurons of origin of the aberrant axons die [46], but in other situations neurons often survive the loss of their aberrant axons [43]. The present interpretation that this small and transient projection is caused by axonal targeting errors could be contested, and it has been argued that it could be a phylogenetic relic [47], but it is generally considered by neurodevelopmental specialists to reflect targeting errors. This interpretation is supported by the great variability in the number of ipsilaterally projecting axons [46]. More experiments are needed to test whether other aberrant projections are numerically variable, and ideally this would be done in isogenic or inbred strains. A second kind of putative error in this projection is that ION neurons projecting to the ‘correct’ (contralateral) retina often project to the topographically ‘wrong’ part of it. Unlike the rare ipsilaterally projecting ION neurons, these topographically incorrect axons are numerous, accounting for 25–50% of the total isthmo-optic projection. They are also eliminated during the ION’s period of naturally occurring neuronal death, and their neurons of origin do indeed die [48].

An important question is how the aberrant axons ‘know’ that they are aberrant and need to be eliminated. Experiments on a variety of systems indicate that axon maintenance depends on spontaneous electrical activity occurring prenatally [49,50], and it has been argued that this could eliminate aberrant connections by a Hebbian-like mechanism that strengthened only synapses that were successful in activating the postsynaptic neuron [51].

Thus, the formation of brain connections requires trial-and-error testing all along the axonal growth pathway, allowing a considerable degree of imprecision in the initial connections, followed by a period of refinement in which most aberrant connections are eliminated by an activity-dependent, probably Hebbian, mechanism that serves as a kind of pre-functional inspection procedure.

4. STOCHASTICITY IN NEURAL ACTIVITY AND ITS EFFECTS ON THE BRAIN
The above-argued roles of intrinsic chance at the level of individual cells (§2) and in the establishment of axonal connectivity (§3) will presumably cause some degree of stochasticity in neural activity. We here address the inverse question of whether stochasticity in neural activity will spread sufficiently to affect the development of cellular biology and brain connectivity.

There is abundant evidence that the brain’s electrical activity has profound effects on the brain, beginning very early in development (long before birth in mammals) and continuing throughout life. The electrical activity directly affects signalling pathways in neurons (and probably glia) [52] and thereby influences other basic cellular processes such as the transcription and translation of trophic and other molecules [53,54]. During development, this leads to effects on axonal growth, dendritic growth [55], synapse formation and stabilization [56], and neuronal survival [57,58]. In later stages of development and adulthood, the electrical activity modifies synaptic weights and continues to modulate gene expression. The question is whether the resulting stochasticity will spread sufficiently to affect these processes.

(a) Sensitivity to fluctuations in firing pattern
Evidence from two different approaches, single neuron stimulation and chaos theory, indicates that in many situations the stochasticity will spread.
Stimulation of a single neuron in the hippocampal CA3 region modified the overall rhythms in hippocampal CA1 [59], and repetitive high-frequency stimulation of a single cortical neuron triggered a switch between different cortical sleep states [60]. Single neuron stimulation has also been shown to affect behaviour. Provoke a small train of action potentials (spikes) in a single neuron of the somatosensory barrel cortex in conscious rats provoked a small but detectable change in the animal's performance in a detection task [61], although a similar stimulation of the thalamus did not produce a significant effect [62]. All these last-mentioned experiments involved the artificial production of multiple spikes, so the question remains whether a single spike would have any significant effect. This question has been addressed in the cerebral cortex, where every neuron receives and makes a few thousand synaptic connections, most of which are continually firing. It might be supposed that a single additional spike would be lost in the noise (or pseudo-noise) of all the ongoing activity, but this does not appear to be the case, because a combination of electrophysiological experimentation and theoretical analysis indicates that a single extra spike in one neuron produces an additional spike in about 28 of the postsynaptic target neurons [63]. Each of these will presumably evoke another 28 or so spikes at the next stage, and so on, implying that after \(n\) stages of transmission there will be about \(28^n\) extra spikes, all resulting from a single initial one. This amplification mechanism could be problematic for cortical function if it led to runaway activation of the cortex, but there is evidence that average neuronal activity levels are kept more or less constant by a variety of homeostatic plasticity mechanisms, including adjustments of synaptic strengths and of ion channel expression levels [7]. An important site of this plasticity appears to be the fast-spiking parvalbumin-positive inhibitory interneurons [64]. But preventing overactivation is a different question from the prevention of noise-propagation, which might still occur even in a system whose overall level of activation remained stable. The separate question of noise-resistance is dealt with in §5.

Chaos theory provides an independent approach to the same question of sensitivity to fluctuations. Chaotic systems are extremely sensitive to perturbations. Since the 1980s, numerous electrophysiological studies of brain activity have been interpreted as evidence for chaotic processes [65]. It is technically very difficult (perhaps impossible) to prove rigorously from a series of action potentials or waves recorded from the brain that the underlying process is truly chaotic, but the current consensus is that chaos often occurs in brain activity [66,67].

Thus, two different approaches indicate that small fluctuations in brain activity may be amplified and propagate, causing potentially causal changes in neural circuits.

(b) Sensitivity to spike timing

The interaction between neurons in complex structures like the cerebral cortex has been likened to that between musicians in an orchestra; they must precisely orchestrate their neural activity to function adequately [68]. This need exists also at the level of individual neurons, because most neurons need to be activated almost simultaneously by several inputs to produce an action potential, and they are very sensitive to tiny variations in the synchrony of the inputs. Such sensitivity may not be unique to neural activity, because synchronous activation of various biochemical pathways can be required for certain effects to occur [69], but the temporal resolution of the synchrony can be more than a million times greater for generation of an action potential [70].

Spike timing is also important for synaptic plasticity and the phrase 'spike timing-dependent plasticity' (STDP) is in standard use. Numerous papers published over the last two decades have established the occurrence of approximately Hebbian STDP in several regions of the brain [71], according to which the efficacy of a synapse is increased if a presynaptic action potential occurs immediately before the firing of its postsynaptic target neuron. Indeed, most long-term potentiation (LTP) is established according to a Hebbian rule. But the same rule is believed to be applied to the development of neural connections, and in this context, it led to the slogan 'neurons that fire together wire together'. It is not so much the initial formation of connections as their subsequent stabilization that depends on a Hebbian mechanism. According to Hebb's rule, synapses would be strengthened only when the postsynaptic firing occurred immediately after the presynaptic potential, and indeed it is now known that LTP occurs only if this condition is respected, and the opposite effect of long-term depression (reduction in synaptic efficacy) occurs if the order is reversed [72]. Work on cultured hippocampal neurons [73] showed that a change of as little as 5 ms in relative spike timing could transform synaptic enhancement into synaptic depression.

It may thus be concluded that a variety of neural events occurring during development and later, including synaptic stabilization and synaptic plasticity, are critically sensitive to the synchrony of spike timing between different neurons. Therefore, interneuronal differences in spike timing are likely to be a significant form of intrinsic chance in brain development and throughout life.

5. NOISE RESISTANCE

As noise occurs at every level, from that of molecules to that of neural circuits, one would expect organisms to have developed strategies of resistance to noise, and there is abundant evidence that this is the case. Some strategies of noise resistance, such as buffering it by redundancy or negative feedback, are intuitively obvious, but in complex situations, detailed mathematical analysis and/or computer simulation may be required to understand whether a system will be resistant or vulnerable to noise.

(a) Noise resistance in biochemical circuits

The omnipresence within cells of chance events such as thermal fluctuations has stimulated considerable interest in the problem of noise resistance among cell biologists, and numerous mechanisms of noise resistance in intracellular circuits have been studied [74].

The simplest of these is mass action, and it is a major cellular strategy to compensate for the chance aspects of molecular diffusion. For the formation of chemical complexes between diffusible molecules (including the docking of substrates to enzymes, and the binding of ligands to receptors, etc.), individual molecules are usually redundant in the sense that many others are
available to replace them. Thus, randomness in the behaviour of a given molecule will tend to be averaged out over the many other molecules involved.

Redundancy is another source of noise resistance. A simple example of this is provided by the fact that we have two alleles of each autosomal gene. If one allele is deleted, or fails to transcribe, or encodes a malfunctioning protein, the consequences are often minor because the gene products of the other allele are sufficient for the functional requirements of the cell. In some cases, the deletion of an allele causes a twofold reduction in the amount of gene products, but in situations where the precise amount is important, there is usually a negative feedback mechanism to maintain it at the appropriate level. Gene duplications are another kind of genetic redundancy. They are widespread throughout all known genomes. Duplicated genes are never identical but are homologous, and often have considerable functional redundancy. One of the consequences is a great increase in the robustness of organisms to mutations and gene deletions, presumably because the gene products of the intact gene(s) can compensate for the loss of their homologues. Buffering by redundancy is usually not the only function of gene duplications, and the homologous gene products are often temporally or spatially distinct in their expression patterns, implying differences in function. Nevertheless, in many cases, the loss of one of the duplicated genes can be compensated for by the upregulation of the other [75].

Negative feedback is ubiquitous in cells, and is widely considered to be a means of reducing noise. There is an important exception to this; if there is noise within the feedback path, the feedback will make the system still more noisy, not less. Nevertheless, there is theoretical and experimental evidence that negative feedback in intracellular pathways does in many cases reduce noise [25].

Negative feedforward mechanisms, involving microRNAs, can also in some cases confer noise resistance by preventing ectopic protein molecules from appearing inappropriately or by buffering fluctuations in expression levels [76].

(b) Noise resistance in neural circuit refinement
Noise-resistance mechanisms also operate at the level of neural circuits. As discussed in §2, the importance of aberrant axon growth appears to be diminished by the subsequent refinement of neural connections through synaptic plasticity, axon pruning and sometimes neuronal death. This circuit refinement is in itself a form of error-correction or noise-resistance.

(c) Circuits whose outputs vary less than the circuit components
Biologically realistic theoretical models indicate that, under some circumstances, large variations in circuit parameters can have minimal functional consequences. This was shown strikingly in a simulation of a crustacean stomatogastric ganglion [77], and is supported by cell biological data on mammalian neurons, but it is unclear whether such stable-output devices are found in mammalian brain circuits, whose activity can be rather sensitive to minor fluctuations in their components, as is discussed in §4.

(d) Noise-tolerant strategies of self-organization
The above discussion emphasizes noise resistance by reducing the noise or its effects (e.g. by negative feedback). But the organism also employs the strategy of adopting self-organization algorithms that render moderate noise simply irrelevant to the global result. Self-organization is the process by which a globally coherent pattern appears as a result of purely local interactions between the elements involved, and this is believed to be a major contributor to neural (and general) development. It would be beyond our present scope to review the vast field of neural self-organization, so I here discuss just one example of noise-tolerance in a computational model of self-organization in the visual system.

Linsker showed, in a simple multilayered artificial network with initially random feedforward connections and spontaneous electrical activity, that Hebbian synaptic modifications could promote spontaneous self-organization highly reminiscent of what happens in the developing visual systems of cats and monkeys. First, opponent centre-surround receptive fields appeared for artificial neurons in the lower layers of the system, resembling the receptive fields of retinal ganglion cells and lateral geniculate neurons [78]. Then, cells with oriented receptive fields resembling those of visual cortical neurons developed in higher layers [79], and in the presence of lateral connections between developing orientation neurons, they self-organized into banded patterns resembling cortical orientation columns [80]. The relevance of this to the question of noise resistance is that all these features develop reliably from randomly chosen initial conditions, implying that noise would not disrupt the global development of the system to give centre-surround fields at low levels and oriented ones arranged in columns at higher levels. That is not to say that the details would be unaffected by noise. For example, the decisions of which ‘neuron’ is to have a vertical receptive field and which one a horizontal field might be affected, but this would probably not matter for the overall functioning of the system so long as all orientations were represented. Similar noise tolerance is found in other models of neural self-organization [81].

(e) The resultant of noise, noise amplification and noise buffering
To summarize, many cellular events and intercellular interactions are incompletely controlled by the combination genes-plus-environment. At the molecular level, these include molecular movements contributing to diffusion and thermally induced vibrations, which cause variability in many cellular events including gene expression and ion channel function. At the level of cell-to-cell interaction, there is additional stochasticity in neurite growth and in the synchrony of spike-timing in interacting neurons, which will in turn affect synaptic stabilization and plasticity, hence connectivity and neural functioning (figure 1).

The importance of these noise phenomena for brain functioning is difficult to evaluate, because some cellular and neural mechanisms buffer the noise, whereas others amplify it. The success of the noise-buffering mechanisms is apparent from the fact that genetically identical organisms reared in similar environments differ only slightly, but the existence of subtle differences even in these
organisms suggests that the residual noise still has significant effects.

The existence of noise-buffering mechanisms constitutes, in itself, evidence that the noise really does matter. For example, the lower level of gene expression noise for genes that are critical for survival suggests that the noise matters at least for these genes [27]. Likewise, the fact that aberrant axons and axonal branches are eliminated during development suggests that their persistence would have been detrimental had they remained [2]. This implies that stochastic effects serious enough to compromise evolutionary fitness are eliminated or buffered, whereas functionally insignificant ones are likely to remain. I therefore propose that evolution has given us sufficient noise-buffering capacity to preserve functionality, but that this is insufficient to cause exact genes-plus-environment determinism.

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