The biochemical basis of synaptic plasticity and neurocomputation: a new theory

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SUMMARY

The recent finding that dendritic spines (on which 90% of all excitatory synapses on pyramidal cells are formed) are not permanent structures but are continually being formed and adsorbed has implications for the present theoretical basis of neurocomputation, which is largely based on the concept of fixed nerve nets. This evidence would tend to support the recent theories of Edelman, Freeman, Globus, Pribram and others that neuronal networks in the brain operate mainly as nonlinear dynamic, chaotic systems. This paper presents a hypothesis of a possible neurochemical mechanism underlying this synaptic plasticity based on reactive oxygen species and toxic *o*-semiquinones derived from catecholamines (i) by the enzyme prostaglandin H synthetase induced by glutamatergic NMDA receptor activation and (ii) by reactive nitrogen species derived from nitric oxide in a low ascorbate environment. A key factor in this neuromodulation may be the fact that catecholamines are potent antioxidants and free radical scavengers and are thus able to affect the redox mediated balance at the glutamate receptors between synapse formation and synapse removal that may be a key factor in neurocomputational plasticity. But catecholamines are also easily oxidized to neurotoxic *o*-semiquinones and this may be relevant to the pathology of several diseases including schizophrenia. The relationship between dopamine release and positive reinforcement is relevant to this hypothesis.

1. HYPOTHESIS

The present theoretical basis of neurocomputation is the concept that the essential feature of neuronal activity relevant to neurocomputation and the organization of memory and behaviour lies in altering the weights of individual Hebbian synapses in a fixed neural net. An alternative view, based on nonlinear dynamics and chaos theory, is that put forward by Freeman (1994) that the brain operates by flexible patterns of neural activation that may use different particular neurones each time they are expressed. It is only the statistical aspect that counts. Edelman (1994), Globus (1992) and Pribram (1997) have put forward similar ideas. This alternative mechanism was expounded by Roy John (1979) as follows: 'It is not the location of cells that matter, but rather the rhythm at which they fire … cells combine to perform mental functions by a statistical process … '; and by Edelman (1994): 'Decisions in such [selective] systems are based on the statistics of signal correlations.' Pribram in numerous publications has stressed the role that holographic and holonomic processes may play in brain function. Pritchard & Duke (1992) give a good review of this subject. Classical nerve net theory postulates a changing set of weights in the same synapses spread over the relevant parts of the net; that is what the training programs laboriously construct during the training sessions. However, superimposed on this mechanism there may be a second one in which some synaptic modulation may be mediated by the formation of new synapses rather than by strengthening old ones.

The main excitatory synapses in the cortex are glutamatergic, in which the axon terminal is in apposition with a dendritic spine. It may therefore be relevant that recent data indicate that spines are not permanent structures but are constantly being formed and removed as needed (Segal 1995; Cramer & Sur 1995). *N*-methyl-D-aspartate (NMDA) antagonists cause more spines to form on the dendrites of neurones in the lateral geniculate nucleus (Cramer & Sur 1995). In cultured neurones spine numbers increase 6 h after stimulation (Segal 1995). Spine numbers are also affected by such factors as deafferentation, hibernation, oestrous, epilepsy and an enriched environment (Segal 1995; Schauwecker & McNeill 1996; Thompson *et al.* 1996). In schizophrenia spine numbers are reduced by 50% both in the cortex (Garey *et al.* 1995; Glantz & Lewis 1995) and the striatum (Roberts *et al.* 1995). There is also decreased synaptic density in the left thalamus (Blennow *et al.* 1996). In cases of severe mental retardation dendritic spines are almost absent (Harris & Kater 1994). As Bauer (1996) says, 'Once established (probably according to an endogenous genetic program), cortical synapses are not fixed

''hardware'' but are subject to a permanent turnover.' Mirmiran *et al*. (1996) state 'individual neurons compete for a given target (synapse); those synapses that are activated survive and those that are not used are eliminated'; in other words, 'use it or lose it'. However, I present evidence that we should amend this slightly to add the proviso that those synapses that are not activated in the presence of neurochemical signals (e.g. widespread release of dopamine or possibly norepinephrine) mediating the receipt of positive reinforcement by the organism, even if used, will also tend to be eliminated.

Spine creation and destruction at glutamatergic synapses is largely controlled by glutamate itself. Some species of glutamate receptors (e.g. some metabotropic receptors) activate phosphorylation of skeletal microtubular protein and influence synaptic maturation, spine morphology, and possibly the growth of new spines (Harris & Kater 1994). On the other hand, glutamate is also a potent neurotoxin and can, under certain circumstances, produce neuronal damage, and possibly spine elimination. NMDA and kainic acid (KA) receptors activate proteolysis (destruction) of brain spectrin and MAP2, structural components of the spine cytoskeleton (Siman & Noszack 1988; Cramer & Sur 1995; Segal 1995). The mechanism for spine removal may be based on a loss of trophic factors. But it may also be influenced by some neurotoxin. One candidate for this is the following mechanism.

NMDA receptors also activate brain prostaglandin H (PG H) synthase via the cascade: calcium inflow, phospholipase A 2 (PLA2) activation and arachidonic acid release (Yamagata *et al*. 1993; Adams *et al*. 1996; figure 1). PG H synthase is located in neuronal bodies and dendrites including dendritic spines, but not in axon terminals (Breder *et al*. 1992; Kaufman *et al*. 1996; Thore *et al*. 1996). In the striatum (and perhaps elsewhere) PG H synthase has a cofactor, dopamine, which it co-oxidizes to dopamine quinone (Hastings 1995; Mattammal *et al*. 1995), which in turn forms the cascade: aminochrome, the highly neurotoxic dopamine *o*-semiquinone, dopamine hydroquinone, dihydroxyindole and, finally, in some loci, neuromelanin. The synthesis of the *o*-semiquinone leads to the release of large amounts of reactive oxygen species (ROS). ROS, from this source and from other oxidases, play a large role in NMDA and non-NMDA glutamate excitotoxicity (Dykens *et al*. 1987; Coyle & Puttfarcken 1993; Favit *et al*. 1993; Lafon-Cazal *et al*. 1993; Rothman & Olney 1995; Patel *et al*. 1996). Glutamate is not neurotoxic in an anaerobic environment (Dubinsky *et al*. 1995), and its toxicity is inhibited by antioxidants (Ciani *et al*. 1996; MacGregor *et al*. 1996). Dopamine toxicity is mediated by ROS and its *o*-quinone derivatives (Michel & Hefti 1990; Cadet & Kahler 1994; Ben-Shacher *et al*. 1995; Ohmori *et al*. 1996) acting, not on DA receptors, but on NMDA glutamate receptors, leading either to cell death or inhibition of neurite growth (Lieb *et al*. 1995). The apoptosis triggered by dopamine can also be prevented by antioxidants (Offen *et al*. 1996). The neurotoxic effects of glutamate are also mediated by other complex mechanisms based on ROS formation, e.g. (i) inhibition of the mitochondrial electron transport chain by depolarization of the mitochondrial membrane by activation of the permeability transition pore (PTP), which drains ATP reserves (White & Reynolds 1996); (ii) inhibition of the glutamate reuptake mechanism (Trotti *et al*. 1996); (iii) increased glutamate release (Pellegrini-Giampietro *et al*. 1988); (iv) depressing glutamine synthesis by oxidative damage to the enzyme glutamine synthase, thus leading to increased levels of glutamate (Oliver *et al*. 1990); and (v) inhibition of cystine uptake leading to glutathione deficiency (Kato *et al*. 1992). Norepinephrine toxicity is also mediated in part by its oxidized *o*-quinone derivatives and ROS (Louis *et al*. 1992).

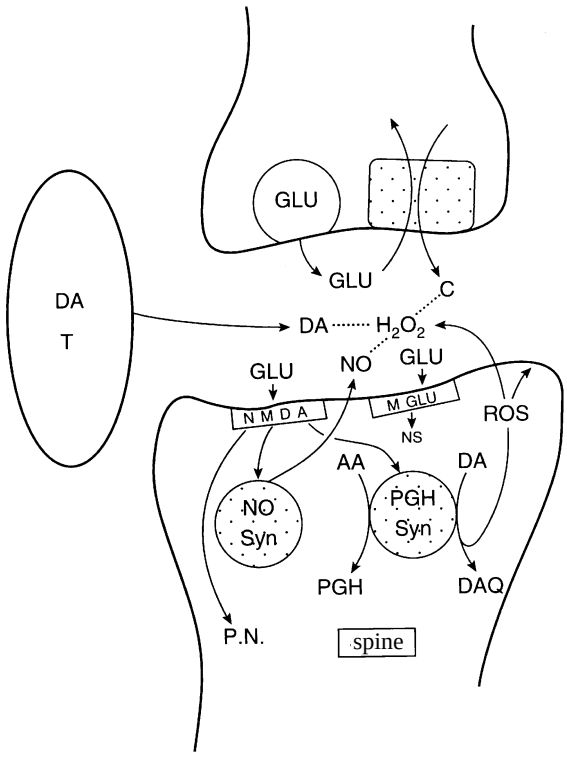

Figure 1. Diagram of the glutamate receptor. AA, arachidonic acid; C, ascorbate; DA, dopamine; DA T, dopamine terminal; DA Q, dopamine quinone; M GLU, metabotropic glutamate receptor; NO, nitric oxide; NO Syn, nitric oxide synthetase; NMDA, *N*-methyl-D-aspartate glutamate receptor; NS, neuroskeletal enzymes; PGH, prostaglandin H; PGH Syn, prostaglandin H synthetase; PN, proteases and nucleases; ROS, reactive oxygen species.

The possible importance of this pathway is indicated by the fact that dopaminergic neurones (cell bodies in the substantia nigra and ventral tegmental area, together with their axons in the striatum) contain the enzyme DT-diaphorase, which converts the dopamine-derived toxic *o*-semiquinone to the *o*-hydroquinone, which can be further metabolized to non-toxic products (by catecholamine-O-methyl transferase (COMT) and sulphotransferase) (Schultzberg *et al*. 1988; Segura-Aguilar 1996). DT-diaphorase is also found in the cortex, but here mainly in glia. The presence of this enzyme in dopaminergic neurones suggests that these may produce *o*-semiquinones against which protection is needed. However, it must be noted that an

established function of DT-diaphorase in the membrane is to act as a two-electron reductase to maintain the antioxidant (reduced) form of coenzyme Q (Beyer *et al*. 1996). The conversion of the relatively non-toxic hydroquinone to the toxic *o*-semiquinone is normally inhibited by the antioxidant enzymes catalase and superoxide dismutase (Segura-Aguilar & Lind 1989; Baez *et al*. 1995).

In certain brain areas the spines on the medium spiny output neurones have two contacts, one a glutamatergic terminal on the spine and the other an adjacent non-synaptic dopaminergic (DA) terminal *en passage*. These two terminals are often only 1–2 μm apart (Kötter 1994). Catecholamines are potent antioxidants and free radical scavengers (Liu & Mori 1994). These authors suggest that this may play an important role in brain function. Thus it is possible that catecholamines from the *en passage* DA bouton (and possibly norepinephrine (NE) boutons in NE systems) may modulate the redox status of the adjacent glutamatergic synapse by scavenging ROS. Kullman *et al*. (1996) have presented evidence that NMDA receptors on one cell can be stimulated in a similar way by glutamate released by axon terminals on neighbouring cells: they call this the 'spill-over' effect. Sesack & Pickel (1990) also found that DA and glutamate can interact by non-synaptic mechanisms 'perhaps following diffusion from synaptic sites of release'. H_2O_2 can diffuse out of the post-synaptic neurone into the synaptic cleft and there, under certain conditions, undergo conversion to superoxide and hydroxyl ion radicals. Edelman & Gally (1992) have suggested that H_2O_2 might have a modulatory role in synapses. There is direct evidence that dopamine protects against glutamate neurotoxicity (Amano *et al*. 1994) but this may be due in part to the fact that activation of DA receptors inhibits glutamate release (Olney & Farber 1995).

Activation of the glutamate NMDA receptor also activates nitric oxide (NO) synthase. NO has two redox forms, the toxic nitrogen monoxide radical $NO^{\bullet}$ (which forms peroxynitrile) and the neuroprotective nitrosium ion NO^{+}. The neuroprotective effect of NO may be mediated by scavenging ROS, in particular hydroxyl ions (Lancelot *et al*. 1995), or by inhibiting DA reuptake by a direct effect on the DA reuptake mechanism (Cook *et al*. 1996), or by evoking DA release (in the striatum) and NE release (in the cortex) (Peterson *et al*. 1995), which, in the presence of adequate antioxidant cover, would increase the antioxidant defences of the synapse.

The NMDA receptor has a redox modulatory site containing -SH group(s) that modulates the activity of the NMDA receptor. Oxidation of this redox site by thiol oxidants leads to down-regulation of the NMDA receptor and its reduction by DTT has the opposite effect (Aizenman *et al*. 1989). In which case one might expect that ROS, if they too oxidized this site, should have a neuroprotective rather than a neurotoxic effect. Lipton *et al*. (1993) claim that NO exerts its neuroprotective effect by oxidizing these -SH groups, and so down-regulating the NMDA receptor, leading to decreased ROS production. However, Dawson & Dawson (1996) claim that NO inhibits NMDA-induced currents by interacting with cations rather than with the redox site on the NMDA receptor. In fact, Dawson *et al*. (1991) report that NO synthase inhibitors prevent glutamate neurotoxicity, in which case NO would mediate glutamate neurotoxicity rather than preventing it. Furthermore, NO directly interacts with and enhances PG H synthase activity, which would result in greater ROS production (Salvemini *et al*. 1993). Also, NO, in the absence of adequate antioxidant cover (particularly ascorbate), can form reactive nitrogen species (RNS) that can oxidize dopamine to toxic *o*-semiquinones (Cook *et al*. 1996). Thus, clearly the interaction of glutamate, dopamine, NO and ROS is very complex. Under ordinary circumstances the main antioxidant defences at the glutamate receptor would appear to be ascorbate, catecholamines and NO.

The importance of the redox state of the glutamate receptor may explain why the transport proteins that take up glutamate from the synapse do so in exchange for ascorbate (Rebec & Pierce 1994). Ascorbate is the principal antioxidant in the brain and would serve to scavenge the ROS generated by glutamate receptor activation and also protect dopamine against oxidation by RNS derived from NO. However, there is as yet no direct, as opposed to indirect, evidence that reactive oxidized dopamine metabolites specifically attack the NMDA receptor. Even if they do, the data of Aizenman *et al*. (1989) make it unlikely that their target would be the redox site on the NMDA receptor. More work on the physiology and pharmacology of these compounds is needed.

Catecholamines are clearly double-edged weapons. Their antioxidant properties render them neuroprotective. On the other hand, the fact that they can easily oxidize to form highly neurotoxic *o*-semiquinones renders them potentially neurotoxic. The balance between these two is maintained by a very complex mechanism in which ascorbate, NO, and the mechanisms that prevent formation of *o*-semiquinones (e.g. COMT, sulphotransferase, PD-diaphorase, superoxide dismutase and catalase, 5-cysteinylization and 5-glutathionization (Segura-Aguilar *et al*. 1996) all *inter alia* play a role.

This hypothesis suggests that the delicate balance at the glutamatergic synapse between synapse formation (by the actions on the cytoskeleton listed above) and synapse elimination (by ROS and in some loci possibly catecholamine *o*-semiquinones) could be modulated by the antioxidant effect (ROS scavenging properties) of the catecholamines, thus tipping the balance in the direction of spine formation and preservation. This would tend to eliminate those glutamatergic synapses, which were not activated in conjunction with the simultaneous release of those catecholamines (such as dopamine), that are thought to mediate signals indicating the receipt by the organism of positive reinforcement, and to promote those synapses that were. This mechanism could play a role in learning and neurocomputation, an example of neural constructivism or Darwinism at work. However, some malfunction of this mechanism might carry the risk of

generating excessive amounts of neurotoxic ROS and neurotoxic catecholamine-derived *o*-quinones that may play a role in some neurodegenerative disorders, such as schizophrenia and epilepsy (Smythies 1996*a*, *b*; Smythies *et al*. 1997).

It is currently thought that neuromodulators exert their effects solely via their own specific receptors. However, some of these, such as catecholamines, may produce some of their effects on other receptors (e.g. some glutamate receptors) by a 'spill-over' effect and redox reactions of the type described in this paper.

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