Dynamic and Stochastic Behaviour of Neocortical Synapses.

A thesis submitted to the University of London in part fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Life Sciences.

By

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DECLARATION

This thesis describes research conducted in the School of Pharmacy, University of London between 2005 and 2010 under the supervision of Professor Alexandra M. Thomson. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

Signature

Date: 27/02/2012
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The work presented in this thesis benefited from a wealth of contributions and interactions that now feels nearly impossible to track down exhaustively. Notwithstanding, but with apologies for unforgivable omissions,

In any case, a single page can not possibly accommodate the expression of my kaleidoscopic indebtedness and gratefulness towards Prof. Alex Thomson, supervisor extraordinaire. I shall therefore make the most of the occasion, and indulge in this florid writing style, the necessary repression of which she single-handedly endeavoured to accomplish from the rest of this manuscript, in the hope to demonstrate absolute sincerity in the aforementioned. Endless thanks, more specifically, for sharing tremendous expertises and the enduring unconditional support.

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ABSTRACT

The neocortex receives inputs from many other brain regions, it contains many different types of neurones in 6 layers and processes large volumes of information. This thesis deals with some of the properties of the local synaptic circuitry of the neocortex.

Dual intracellular recordings with biocytin labelling were performed in slices of adult rat neocortex in vitro using conventional sharp micro-electrodes. Responses of postsynaptic cells to trains of presynaptic action potentials were recorded. Histological processing identified the cells recorded and laminar location.

The amplitude of each excitatory postsynaptic potential (EPSP), in each sweep was measured. Subsets of measurements for which conditions were deemed to be stable were selected. For recordings that included multiple data subsets whose amplitudes differed primarily because of differences in presynaptic release probability (p), the binomial parameters n (number of synapses), p and q (quantal amplitude) were estimated by fitting relationships between EPSP coefficient of variation, variance or proportion of failures of release and mean amplitude, with equations based on simple binomial models. Striking differences in the binomial parameters estimated for different classes of connections were found.

To determine how far the outcomes of this analysis depended on the assumption of a simple binomial model in which p and q are identical at all synapses, Monte-Carlo simulations of simple and more complex binomial models of synaptic release were generated. These models demonstrated the wide range of conditions under which analysis based on simple binomial models can provide reliable estimates of n, p and q.

Computational models (NEURON) that integrate short term synaptic dynamics with a stochastic simulation of synaptic transmission were developed. These models display properties similar to those displayed by synapses, but not observed in traditional deterministic models of release. For example, recovery from synaptic depression has peaks and troughs superimposed on a smooth exponential decay.
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ABBREVIATIONS

A Ampere, unit of electrical current
ABC Avidin-Biotin-HRP Complex
ACSF Artificial Cerebro-Spinal Fluid
Ad hoc Adequate to a particular purpose
-Agatoxin IVA Peptide inhibiting P- and Q-type calcium channels
Ampl. Mean amplitude
AnalaR Chemical reagent of high purity
AP Action potential
AXOPROBE 1 Amplifier
bad Burst-adapting characteristics
C Carbon
Ca, Ca²⁺ Calcium, calcium ion
CA1, CA3 Cornu Ammonis area 1, Cornu Ammonis area 3
CC, cc Cortico-cortical
CED 1902 Cambridge Electronic Design, signal conditioner
CED 1401 Cambridge Electronic Design, analog to digital converter
c+[unit] "Centi-", prefix, factor 10⁻²
Cl, Cl⁻ Chloride, chloride ion
Cm Specific capacitance, f.cm⁻²
CNS Central Nervous System
-Conotoxin GVIA Peptide blocking L- and N-type calcium channels
corr. Correlation
CT, ct Cortico-thalamic
CV Coefficient of Variation
Da Dextrogyre
Da Dalton
DAB 3,3 Diaminobenzidine tetrahydrochloride
e Unspecific postsynaptic current
e.g. Exempli gratia, "for example"
EPSP Excitatory Post-Synaptic Potential
et al. Et alii, "and others"
f Farad, unit of electrical capacitance
F Failure rate
g Gramme, unit of weight
g Postsynaptic conductance, theory
GABA Gamma Amino Butyric Acid
Glut. Glutamate
H Hydrogen
HRP Horse Radish Peroxidase
HW Half-width
Hz Hertz, unit of frequency, number of cycles (continuous signal) or occurrences (discrete) per second
i Postsynaptic current, theory
i.e. Id est
iBF Intrinsic burst firing
IPSP Inhibitory Post-Synaptic Potential
ISI Inter-Spike Interval
k+[unit] "Kilo-", prefix , factor 10^3
K, K+ Potassium, potassium ion
K, k Number of quanta released
KS Kolmogorov Smirnov
L+[1-6] Neocortical layer one to six
L Litre, unit of volume
m+[unit] "Milli-", prefix , factor 10^-3
M+[unit] "Mega-", prefix , factor 10^6
M Molar, unit of solute concentration, mole.L^-1
M Mean amplitude
Mg Magnesium
m Metre, unit of distance
mb. Pot. Membrane potential
min Minute, unit of time
mol Mole, unit of quantity
MW Molecular weight, g.mol^-1
n+[unit] Nano, factor 10^-9
'n' Number of available quanta of transmitter
n Number of release sites
Na, Na+ Sodium, sodium ion
Ni Nickel
NSF N-ethylmaleimide-sensitive factor
O Oxygen
O^2/CO^2 Carbogen
P Phosphate
P Probability of obtaining a particular value of a test statistic assuming the null hypothesis is true.
'p', p Release probability
p0 Initial release probability, i.e. probability at rest
PB Phosphate buffer
PBS Phosphate Buffer Saline
PPR Paired pulse ratio
PSP Post-Synaptic Potential
p&q Complex binomial model of release
'q', q Quantal size
Ra Axial resistivity, Ohm.cm
RT Rise time
r^2 R-square, coefficient of determination.
s  Second, unit of time
S  Siemens, unit of electrical conductance
S  Sulfur
S1  Primary somatosensory cortex
SNARE  Soluble NSF Attachment protein Receptor
Spike 2  Signal acquisition software
UK  United Kingdom
USA  United States of America
V  Volt, unit of electric potential
V  Postsynaptic membrane potential, theory
V  Variance of amplitude within a subset of EPSPs
V/M  Variance over Mean Amplitude
V1  Primary visual cortex
Var  Variance
w/v  Weight to volume ratio
wpc  Within a probability condition
y  Inactive state variable
z  Recovered state variable
C  Degree Celsius, unit of temperature
µ+[unit]  Micro, factor $10^{-6}$
ω  Omega
Ω  Ohm, unit of electrical resistance
%  Percentage
±  Plus-minus
[#1]:[#2]  Ratio of [#1] over [#2]
σ  Standard deviation
√  Square root
τ  Time constant
1.0 INTRODUCTION

1.1 SYNAPTIC RELEASE MECHANISMS

1.1.1 Outline of fast chemical transmission

Action potentials (AP) generated at the axon hillock, are regenerative waves of depolarisation actively propagated down the axonal plasma membrane. At the active zone, this depolarisation triggers the transient opening of voltage-gated calcium channels. The consequent entry of calcium ions into the terminal causes the fusion of primed vesicles with the plasma membrane lipid bilayer (Fernandez-Chacon and Südhof, 1999; Benfenati et al., 1999), via a specialised molecular machinery. The fusion of the neurotransmitter-filled vesicles establishes a pore through which these molecules are liberated into the synaptic cleft. This process is called exocytosis.

The specific anatomical arrangements at central synaptic contacts align the presynaptic active zone with the postsynaptic density in close apposition. The cleft between these elements is about 20 nanometres wide (Schikorski and Stevens, 1997; Zuber et al., 2005; Rollenhagen et al., 2007); the width being determined by intracleft protein scaffolding (reviewed in Yamagata et al., 2003). By diffusion, the molecules of transmitter reach and bind to specific receptors situated on the postsynaptic face. This binding causes, through conformational changes in these transmembrane protein complexes, the transient opening of a pore selective for specific ion species. The resulting flow of charged molecules across the postsynaptic plasma membrane induces changes in its polarity and resistivity. Such local dendritic changes can then be propagated along portions of the dendritic cable, or
influence later inputs locally, by combinations of passive (Rall, 1964) and active (Sjostrom et al., 2008) mechanisms. The parallel spatial and temporal integration of signals from such inputs over the dendritic tree, combined with intrinsic properties of the postsynaptic excitable cell will thereafter determine its own action potential generation.

Rapid clearance of the transmitter from the synaptic cleft after exocytosis occurs by diffusion and/or neuronal or astrocytic uptake through transmembrane transporters, insuring the transitory character of fast transmission at chemical synapses. The prevalent retrieval modality of vesicles after exocytosis at central synapses remains, in contrast, the matter of a “long-running” debate (LoGiudice and Matthews, 2006; Harata et al., 2006; Granseth et al., 2006).

1.1.2 Functional pools of neurotransmitter

Classical studies establishing the quantal nature of synaptic transmission coincided in time with the first ultrastructural observations of synaptic discontinuity and vesicles (De Robertis and Bennett, 1954, 1955). Experimental demonstrations that these vesicles did store the chemical transmitters (Toll et al., 1977) including the excitatory transmitter glutamate (Curtis and Davis, 1962; Naito and Ueda, 1983; Storm-Mathisen et al., 1983) and released by exocytosis (Heuser et al., 1979) took a further 20 years.

In the order of 5-20 synaptic vesicles can commonly be seen bound to the active zone of central synapses, while up to hundreds of others lie clustered in its vicinity (Fernandez-Chacon and Sudhof, 1999). At fast synapses, vesicle fusion happens extremely rapidly (under 200 μs; Llinas et al., 1981) during the local AP-triggered influx of calcium. This indicates that the molecular interactions involved in vesicle docking and development of
fusion-competence must happen before the AP reaches the terminal (Thomson, 2000a).

Multiple functional pools of transmitter can be defined, pools that find structural correlates in the organisation of synaptic vesicles within the terminal (Fernandez-Chacon and Sudhof, 1999), between which equilibria are molecularly driven (see Benfenati et al., 1999). The immediately available pool (Auerbach and Bennett, 1969) corresponds to vesicles that are docked to the plasma membrane at the active zone, fully release-competent and not retarded (Thomson, 2000b). Vesicles of the readily releasable pool (<15% of total) include those that are docked, fusion-competent but held in stasis by protein-protein interactions, those that are docked but not yet fusion-competent, and yet undocked cytoplasmic vesicles. Finally, the reserve pool contains the majority of synaptic vesicles, tethered to microfilaments of the cytoskeleton and not immediately available for docking. These can be mobilised into the releasable pool under appropriate conditions.

Fine structural correlates of these pools have been identified, like molecular tethers connecting vesicles to microtubules and actin filaments (Hirokawa et al., 1989), connectors linking vesicles together (Fernández-Busnadiego et al., 2010), particles specific to those clusters of active zone material that do accommodate docked vesicles (Phillips et al., 2001) and the related filaments of yet unidentified molecules (Sollner et al., 1993; Zampighi et al., 2006; Sikou et al., 2011) possibly participating in organising vesicle-delivery (but see Shtrahman et al., 2005) to these sites (Landis et al., 1988; Sikou et al., 2007), and hemifusion of docked vesicles (He et al., 2006; Zampighi et al., 2006; Lisman et al., 2007).
1.1.3 Calcium microdomains and vesicle fusion

Freeze-fracture studies indicated that a release apparatus composed of several interlinked molecular components accompanies docked vesicles (Harlow et al., 2001). Its dynamic assemblage imposes particular spatial relationships via the structural linkage between the vesicular, cytosolic and plasmalemmal proteins of the fusion-core complex as well as the calcium channels themselves (Stanley, 1997; Lisman et al., 2007). Accordingly, the distance between the calcium channel particle and the exocytotic pore appears restricted to about 25 nm (Pumplin et al., 1981; Heuser et al., 1979; Robitaille et al., 1990), thereby limiting the distance from calcium entry to calcium-binding sites (Stanley, 1993). This structural constraint participates to the high speed and tight coupling to calcium entry at which release is elicited.

The majority of calcium channels coupled to fast neurotransmitter release at hippocampal and cortical nerve terminals are high voltage-activated N-, P/Q- and/or R-type (Meir et al., 1999; Seagar et al., 1999). Synaptic vesicle fusion requires internal calcium concentrations in the order of 50-100 μM (Adler et al., 1991; Yamada and Zucker, 1992; Llinas et al., 1992). Such high concentrations are only attained within a micro-domain tens of nanometers wide around the inner mouth of the calcium channel (Simon and Llinas, 1985; Fogelson and Zucker, 1985; Seagar et al., 1999). As few as 200 ions entering through one or two nearby channels are expected to be enough to trigger synaptic release in such configurations (Stanley, 1997; Lisman et al., 2007). One prominent and versatile class of molecular participants to the organisation of active zones of recent interest, are the Rab3-interacting proteins (RIMs), whose rôles appear to include tethering of
calcium channels within the complex and contributing to the docking of synaptic vesicles (Kaeser et al., 2011; Han et al., 2011; Deng et al., 2011).

A power of 2 to 4 relationship is commonly found between the calcium current entering the terminal upon depolarisation and the consequent probability of transmitter release (Katz and Miledi, 1970; Llinas et al., 1981; Augustine et al., 1985, 1988). Some members of the synaptotagmin family of proteins (Xu et al., 2009; Lee et al., 2010) have been identified as major calcium sensors for rapid vesicular release (Nishiki and Augustine, 2004a, 2004b; Sudhof, 2004). The activation of such sensors appears to require the cooperative (Smith et al., 1985) binding of several (four to five) calcium ions (Dodge and Rahamimoff, 1967a, 1967b) as direct methods since confirmed (Schneggenburger and Neher, 2000; Wolfel and Schneggenburger, 2003). Together these substantiate the importance of the calcium influx in synchronous release, as small changes would have dramatic effects on the release probability (Thomson, 2000b).

Fusion of the synaptic vesicle involves the rearrangement of the core complex and particularly of the SNARE (Soluble NSF [N-ethylmaleimide-sensitive factor] Attachment protein REceotor) proteins that mediate docking and priming by specific interactions between the vesicular (v-SNARE) and the plasmalemmal membrane proteins (t-SNARE) (McMahon and Sudhof, 1995; Tolar and Pallanck, 1998). The activated sensor complex including Ca\(^{2+}\)-synaptotagmin is thus thought to lower an energy barrier imposed on vesicle fusion by displacing the molecule complexin (Tang et al., 2006; Martin et al., 2011) from the SNARE complex, thenceforth allowing the formation of the exocytotic pore by cross fusion of the leaflets of both membranes (Rizo and Rosenmund, 2008).
1.1.4 Postsynaptic determinants of depolarisation amplitude

A large number of interdependent factors can influence the amount of depolarisation induced at the cell body of the postsynaptic cell after exocytosis.

These include, the spatio-temporal profile of the concentration of neurotransmitter within the synaptic cleft, the types of receptor located postsynaptically (via their affinity for the transmitter, kinetics, desensitization properties and ionic selectivity), and the number and density of those receptors for the size of the conductance engaged.

The amount of depolarisation induced locally, further depends on the local membrane resistance and instantaneous electrochemical driving force.

In the vicinity of the postsynaptic zones, coincident inhibitory inputs may also modify the postsynaptic depolarization, affecting its magnitude either by summation or by shunting through their consequent local increase in conductance.

The conduction of the depolarisation down to the cell body then involves the topographic distribution of passive membrane properties such as conductances, resting potential, resistivity, area, and the spatial arrangement of dendritic branching (Branco and Hausser, 2010). Active dendritic conductances may also shape the response.

Combinations of these factors thus hold the potential for non-linear dependence of the somatic depolarisation upon the total amount of synaptic conductance engaged by the transmitter, in many physiological circumstances.
1.1.5 Synaptic vesicle recycling

After exocytosis, synaptic vesicles can be recycled, refilled and readied for subsequent rounds of release (Bittner and Kennedy, 1970; Sudhof, 2004). Multiple pathways have been suggested for this process that, importantly for short term dynamics, are expected to work on different time scales (Betz and Wu, 1995). The classical longest route applies to fully collapsed vesicles, which are retrieved from outside the active zone by clathrin-dependent endocytosis. This recycling mode is thought to complete through endosomal intermediates (Heuser and Reese, 1973; Holroyd et al., 1999).

Most vesicles in most release cycles however, may skip the total fusion and endosomal intermediates and retain their identity (Koenig and Ikeda, 1996; Murthy and Stevens, 1998; Richards et al., 2000), being reused either after or without even leaving the active zone (Klingauf et al., 1998; Pyle et al., 2000; Stevens and Williams, 2000) through cycles which are called kiss-and-run or kiss-and-stay respectively (Ceccarelli et al., 1973; Zhang et al., 2009; Sara et al., 2002). Presumably, the protein and lipid composition of the vesicular membrane is an important factor in determining its fate. This is particularly important regarding synaptic dynamics and cellular economics, as these vesicles could be refilled and reused faster, as well as by mobilising much less energy, than the ones undergoing full collapse.

Kiss-and-run and kiss-and-stay constitute the fast pathways expected to be used preferentially to recycle vesicles rapidly between the immediately and readily releasable pools (Harata et al., 2006a, 2006b; Aravanis et al., 2003; Gandhi and Stevens, 2003), while the clathrin-dependent recycling happens on a much longer time scale (Koenig and Ikeda, 1996, Pyle et al.,
2000, Richards et al., 2000), considering in particular that endosomes are rarely observed in normal nerve terminals (Sudhof, 2004).

Molecularly, a role in efficient vesicle reloading has been suggested recently for the cytomatrix protein Bassoon at central synapses (Hallermann et al., 2010), as a participant in sustained release capabilities at some connections.
1.2 HISTORY OF THE BINOMIAL MODEL

1.2.1 Quantal hypothesis

In the 1950s, Bernard Katz and colleagues, studying end plates potentials (epps) at the amphibian neuromuscular junction, noticed that in some conditions, such as low extracellular calcium, the postsynaptic responses apparently reduced to discrete, all-or-none miniature units of transmission with similar time courses. They used the term "quantum" to describe the release of chemical that triggered these events.

This allowed epps to be conceived as composite multiples of the miniature potentials each elicited by the release of one quantum of transmitter. Hence, the trial to trial variation in epp amplitude could be assessed statistically through the assumption that a population of \( n \) units were capable of responding to a nerve impulse with an average probability \( p \), and each responding unit eliciting a miniature epp via one quantum of transmitter \( q \).

This model of synaptic transmission thus entailed that the amplitude of epps would follow a binomial distribution, presenting discrete steps in amplitudes from the occurrences of responses that would involve integer numbers of the \( n \) units.

The adequacy of the model however, was first indicated using its limiting case of the Poisson distribution. In effect, early studies recognised that this simpler case was appropriate when considering a large population of units with a low average probability of response (low calcium conditions). This hypothesis was supported by the strong correlation found between two independent estimates of the mean number of units responding, obtained by measuring the spontaneous miniature potential amplitude on one hand and the failure rate on the other. Further, epp amplitude distributions could be
fitted adequately using a combination of the Poisson series corresponding to these estimates and the parameters of the Gaussian curve fitted to the distribution of spontaneous potential amplitudes (Del Castillo and Katz, 1954; Boyd and Martin, 1956).

In conditions insuring a higher average response probability, the distribution of epp amplitudes obtained from an otherwise identical population of units was thus expected to follow the distribution predicted by the binomial model. Statistically a higher release probability was already expected to provide lower variance. Experiments however indicated fluctuation levels lower than was predicted on that basis alone. Two factors were identified as candidates for such discrepancies with regards to the simple binomial model, the non-linear summation of the potentials due to their correlative increase in end-plate membrane conductance (Martin, 1955), and non-uniform population of units regarding response probability (Brown et al., 1976).

Such fluctuation analysis has since been applied to provide estimates of the Poisson, or binomial parameters „n” (number of quanta available for release), „p” (the probability that each will be released) and „q” (the quantal amplitude, i.e. the amplitude of the postsynaptic response to a single quantum of transmitter) in many physiological studies (Quastel, 1997; Bennett and Kearns, 2000; for discussion).

1.2.2 Applications in the CNS

With technical advances in intracellular recordings, preparations were developed that allowed access to cells in the central nervous system (CNS), like spinal motoneurones of the cat (Brock et al., 1952), toad spinal motoneurones (Kuno, 1957), cortical neurons (Li, 1959) and cat Renshaw cells (Eccles et al., 1961). Early attempts to explain excitatory postsynaptic
potential (EPSP) amplitude-distributions with quantal theoretical models were prevented by the apparent heterogeneity in the characteristics of the responses which was expected to stem from heterogeneity in the dendritic electrotonic locations of synaptic contacts (Katz and Miledi, 1963).

When stimulation conditions were designed to recruit a strictly limited number of monosynaptic afferent fibres to cat spinal motoneurones (histologically demonstrated to terminate within a limited electrotonic range from the soma, [Szentágothai, 1958]), it was found that the fluctuations in evoked EPSP amplitudes would also follow a distribution adequately described by Poisson's law (Kuno, 1964). This provided evidence that the mechanism of monosynaptic transmission onto spinal, and as such central, motoneurones was similar to that in the neuromuscular junction, i.e. monosynaptic EPSPs were built of quantal units of depolarisation with a certain probability of responding upon a presynaptic impulse. In this particular case it was also noted that a single afferent fibre would, on average, release a single quantum at the rate of stimulation that was used.

1.2.2.1 Deconvolution methods and single vesicle hypothesis

Quantal amplitudes at the Group la afferent inputs onto cat spinal interneurones were later inferred through more detailed analysis made possible by progress in recording techniques. Notably, methods were developed allowing inferences from the structure of peaks in the distribution of evoked response magnitudes to be drawn (Edwards et al., 1976; Jack et al., 1981).

These studies substantiated the incremental behaviour of synaptic response magnitudes at central synapses. They did not however, conclude to the adequacy of either a Poisson or simple binomial distribution to
describe experimental distributions. This was suggested to be consistent with failures in presynaptic impulse conduction, nonuniformity in the properties of the release sites (see Brown et al., 1976 for theoretical analysis) and/or postsynaptic response saturation. In particular, fluctuations were proposed to arise from the summation of all-or-nothing quantal transmissions with different magnitudes at the individual boutons constituting a connection (Redman and Walmsley, 1983a, 1983b; Walmsley et al., 1987).

Concomitantly, interneuronal inhibitory synapses on the teleost's Mauthner cell, provided the opportunity to study central synaptic transmission with simultaneous intracellular recordings from the presynaptic and postsynaptic cells (Korn and Faber, 1976), which was coupled with their individual staining (Korn et al., 1981). There, binomial predictions were found to fit the cumulative distributions of fluctuating potentials adequately and the predicted total number of available units was found to correspond to the histologically determined number of active zones. This led to further interpretations that, in this system, under low frequency stimulation, the binomial quantal unit corresponded to the contents of one synaptic vesicle, of which no more than one per release site would undergo exocytosis upon a single presynaptic impulse. This was called the "one vesicle hypothesis" (Korn and Faber, 1991).

Techniques based on fitting amplitude histograms and compound binomial models were used to estimate quantal parameters at mammalian central synapses (Wong and Redman, 1980; Redman and Walmsley, 1983b; Clements et al., 1987; Jack et al., 1990). These approaches relied on the comparison of fits provided by different models of release. The reliability of comparisons between competing models requires a high signal-to-noise ratio and a large sample of post-synaptic responses.
One method of calculating these parameters is to adjust them until an optimal fit to the measured probability density is achieved. The maximum log-likelihood criterion (Hasselblad, 1966) obtained when fitting a model to the measured probability density using the Expectation Maximisation algorithm (Dempster et al., 1977) was used for the comparison of finite mixtures of density functions including quantal and binomial models (Ling and Tolhurst, 1983; Kullmann, 1989, 1992). This approach also provided the basis for the design of a procedure for the hierarchical comparison of increasingly constraining models of release (Strieker and Redman, 1994). The number of active release sites could thereafter be derived from the maximal quantal content “determined as a parameter from the best fitting model rather than from the maximum evoked amplitude” (Redman, 1990).

This approach however, when applied in rat hippocampal slices to EPSCs evoked in CA1 pyramidal neurons by extracellular stimulation in CA3, identified a minority of cases demonstrating quantal fluctuations, the fluctuation patterns of which “could not be described by conventional models of transmitter release” (Strieker et al., 1996).

1.2.2.2 Quantal size at single terminals

One approach to establishing the source of variance in quantal amplitude involved the study of miniature (Bekkers et al., 1990; Bekkers and Stevens, 1995; Liu and Tsien, 1995; Murphy et al., 1995) and evoked responses at connections consisting of a single release site (Gulyas et al., 1993; Arancio et al., 1994; Silver et al., 1996; Auger et al., 1998). Such experiments indicated a large variance in miniature amplitude at single release sites, thus supporting the classical interpretation of quantal analysis (Frerking and Wilson, 1996), as intra-site rather than inter-site variance could
then be expected to account for the major part of the total variance in evoked responses.

It remained difficult, however, to ascertain that only a single release site was involved (Korn and Faber, 1991; Jack et al., 1994), notably when extracellular stimulations of afferent fibres were used, as the required consistency regarding presynaptic involvement could not be established. There were also some indications that changes such as a reduction of quantal variance could be expected to take place along development (Wall and Usowicz, 1998).

Another preparation involving a single presynaptic terminal has been extensively studied as an archetype of fast synaptic transmission in the mammalian central nervous system, because of its experimental accessibility. The calyx of Held is a giant presynaptic terminal making an axo-somatic contact on a principal neuron in the medial nucleus of the trapezoid body in the rat brainstem. This allows paired recordings from the pre- and post-synaptic elements to be performed under visual control (Forsythe, 1994; Borst et al., 1995; Takahashi et al., 1996). The adequacy of postsynaptic current fitting by integer multiples of the miniature currents, indicated that the quantum hypothesis established at the neuromuscular junction also applied at this mammalian central synapse (Sahara and Takahashi, 2001).

1.2.2.3 Statistical methods in fluctuation analysis

The large number of release sites and low probability of release, required for Poisson statistics to apply make this model inapplicable at most central synapses. The signal to noise limitations also make quantal resolution difficult in many central neurons (Silver, 2003). However, statistical analysis
can be used to extract information about quantal parameters \( \langle n \rangle, \langle q \rangle \text{and} \langle p \rangle \) from the amplitude fluctuations of synaptic responses.

Such alternative approaches to conventional quantal analysis were developed by Clamann et al. (1989), Silver et al. (1998) and Reid and Clements (1999), and called ensemble noise analysis, Multiple-Probability Analysis and the Variance-Mean Analysis respectively. These studies measured evoked synaptic responses recorded under a range of stable release probability conditions. These methods have been applied to central synapses (Clamann et al., 1989; Silver et al., 1998; Reid and Clements, 1999; Oleskevich et al., 2000; Clements and Silver, 2000; Bailey et al., 2006), and widely adopted in studies of synaptic plasticity (Foster and Regher, 2004; Oleskevich et al., 2004; Tyler et al., 2006; Humeau et al., 2007; Yamamoto et al., 2010).

These methods use selective experimental manipulations of synaptic properties, specifically altering the probability of release or quantal size, to obtain model predictions of the functional parameters. These estimates are based on the relationships between the measured statistics of the fluctuating postsynaptic responses across the experimental conditions.

One major benefit of this approach over the amplitude histogram fitting methods being that the collection of data is not restricted to the recording of a very large (several hundreds; Clements, 2003) number of events from a single continuously stable transmission condition. Instead, smaller numbers (20-200) of postsynaptic responses can be sampled from each stable transmission condition over the range of experimentally induced conditions (Clements and Silver, 2000), or successive responses in a train (Quastel, 1997; Scheuss and Neher, 2001).
In the studies presented in this thesis, a methodological framework based on Variance-Mean inspired analysis was designed, that allowed response fluctuations to be explored through the simple binomial model. The statistics used were collected from subsets of responses identified at a range of consistent transmission conditions engendered during the study of short term synaptic dynamics.
1.3 SHORT TERM SYNAPTIC DYNAMICS

The magnitude of postsynaptic responses is dynamically dependent upon recent presynaptic activity at chemical synapses (McLachlan, 1978; Thomson, 2000a; Zucker and Regehr, 2002). Several forms of such dynamics have been identified in conditions of repetitive stimulation. Responses can thus be found enhanced or decreased in a temporal pattern dependent fashion, through mixtures of mechanisms picked from a limited palette. The cellular and molecular determinants of these phenomena however, remain largely unknown.

1.3.1 Facilitation

Facilitation corresponds to the enhancement of responses manifested on the hundreds of milliseconds time scale (Markram et al., 1998; Reyes et al., 1998), and faster still (decay time constant ($\tau_{\text{decay}}$) ~ 30–40 ms) in the adult neocortex (Thomson, 1997). The capability for the expression of facilitation appears ubiquitous at neocortical and hippocampal synaptic connections, but it is most clearly apparent in conditions of relatively low release probability (Zucker, 1989; Dobrunz and Stevens, 1997; Thomson, 2000a).

The locus of response-enhancing dynamics is indicated to be presynaptic by quantal analysis, and accompanied by increases in the parameters „p“ and/or „n“ (Fisher et al., 1997). In the case of paired-pulse facilitation these can be envisaged as occurring via increases in the sensitivity of the exocytotic machinery (see section 1.1.3) or in the number of release-ready vesicles at the active zone (Rosenmund and Stevens, 1996). The probability of release from a single active zone being the product of the number of release competent vesicles and their release probability.
The current picture regarding facilitation at central synapses considers its symmetrical predicament to a component of synaptic depression, release site refractoriness. Disposition for one mechanism or the other, is conceived to be arbitrated by calcium sensitivity influencing the prevalence of sites undergoing exocytosis at the onset of the stimulation. Thus, at low sensitivity connections, the amount of calcium necessary to trigger release is not reached following the first action potential at most of the sites. These sites are then left in a rapidly receding state of cooperative calcium-priming (Parnas and Segel, 1981; Barton et al., 1983) from which following spikes can trigger release with increased leverage.

1.3.2 Augmentation and potentiation

Augmentation refers to the enhancement of responses with the number of successive presynaptic action potentials (Magleby and Zengel, 1975) at intervals that can be longer (1-10 seconds) than the decay time of paired-pulse facilitation. Augmentation is, however, dependent on significant facilitation developing first (Thomson, 2000a).

The mechanism for augmentation is also calcium-dependent. It might result from a combination of intraterminal calcium accumulation (Katz and Miledi, 1968) during repetitive firing and cumulative priming of release due to calcium binding cooperativity. An alternative or additional calcium-dependent component could be an accelerated transition of synaptic vesicles to fusion competence.

Potentiation, i.e. post-tetanic potentiation (PTP), is a more slowly developing and decaying ($\tau_{\text{decay}}$ tens of seconds to minutes) process than facilitation or augmentation (Hubbard, 1963). It is thought to result from the calcium-dependent mobilization of the reserve pools of vesicles, shifting
functional equilibria towards more numerous release-competent vesicles. Accordingly, potentiation requires relatively high frequency or tetanic firing for sufficient calcium loading of the terminals (Thomson, 2000a). A rôle is suggested for the neuronal proteins synapsins, in particular synapsin I, in neurotrophin-induced potentiation of excitatory synaptic transmission (Llinas et al., 1991; Greengard et al., 1993; Messaoudi et al., 1998), whereby their phosphorylation-dependent tethering of synaptic vesicles to the actin cytoskeleton at the terminal would regulates the proportion of vesicles available for release (Jovanovic et al., 2000).

1.3.3 Depression

Synaptic depression consists of a decrease in the magnitude of the response upon repetitive or sustained activity. All synaptic connections appear to show some form, or degree of depression. Quantal analysis indicates a presynaptic origin for several forms of depression, reflected in a decrease in the binomial estimates \( p \) and/or \( n \), that results in a reduction in the average number of quanta released (Zucker, 1989).

The most widespread mechanism appears to involve the depletion of the release-ready pool of transmitter. In a symmetrical manner to facilitation (see section 1.3.1), the rate of depression is correlated with the probability of release (Liley, 1956a, 1956b), as the degree of depression decreases with lower extracellular concentration of calcium (Murthy et al., 1997; Thomson, 2000a). At cortical synapses this is estimated to imply between five and ten vesicles, subsequently following a kiss-and-stay time course.

Another mechanism can be at play at connections where release sites have a high sensitivity to calcium. Upon a first action potential, the majority of sites would then release, thereby entering a refractory period (term coined by
Betz, 1970) during which further release is depressed, resulting in later APs having a lower impact through the transitorily decreased „n“ (Bennett and Florin, 1974). Release site refractoriness is suggested to account for a large part of the paired pulse depression apparent at cortical pyramid-pyramid connections (Thomson et al., 1993). The recovery from refractoriness ranges from 30-50 ms to more than 100 ms depending on the type of adult cortical connections, and appears much slower at immature synapses (Markram, 1997).

With longer periods of repetitive activation, the readily releasable pool is also depleted, since it takes tens of seconds for each discharged vesicle to return to the pool (see section 1.1.4). Therefore, progressively less vesicles are available and the prevalence of unoccupied release sites increases. Accordingly, „n“ declines until an equilibrium is reached between the rate of vesicular release and the rate of replenishment of the readily releasable pool (Tsodyks and Markram, 1997; Markram et al., 1998).

By contrast, one form of release-independent depression has also been described at specific neocortical synapses, including low „p“ connections, whereby the average second postsynaptic potential amplitude at intervals shorter than 20 ms was smaller than the average first amplitude, irrespective of whether release or failure took place upon the first action potential (Thomson and Bannister, 1999).
1.4 CORICAL MICROCIRCUITRY

The neocortex is the outermost and phylogenetically most recent structure of the brain. In higher mammals, including humans, its relative size appears to have increased in conjunction with the performance of cognitively more demanding behaviours. A stereotypical organisation is found supporting neural processing across the whole cerebral cortex despite the multimodal character of the functions it supports. Thereby, the neocortex is usually described as a vertical succession of horizontal layers between the meninges of the pia (most superficial) and the astrocytes and myelinated fibres of the white matter (deepest). In its primary sensory regions six such layers can typically be described. Each of these laminae accommodates a specific assortment of neuronal cell types intertwined in selective connectivity (Thomson and Lamy, 2007).

Superimposed on this anatomically defined organisation, a functional one is derived from the tendency of cells in a vertical alignment to share response preference in sensory stimulation experiments (Mountcastle et al., 1957; Hubel and Wiesel, 1962). This is referred to as a columnar organisation. Originating from peripheral perception, the putative flow of information within this idealized functional unit is thought to enter primarily through the major thalamo-recipient layer 4, then, following the preferential local projections of its spiny excitatory cells, to reach supragranular layers 2 and 3. The focussed axonal projection of the pyramidal cells present in layer 3 onto layer 5 pyramids provides, from the latter, one path for corticofugal signal conduction toward subcortical targets, while corticothalamic cells in layer 6 provide distinct corticofugal paths (Reichova and Sherman, 2004; Thomson, 2010) as well as feedback projections onto cells in layer 4 (Stratford et al., 1996; Tarczy-Hornoch et al., 1999; Lee and Sherman, 2008).
Approximately 80% of the cells are excitatory, with spine-bearing dendrites and use Glutamate (Glut.) as a fast neurotransmitter. The other 20% are GABAergic (Gamma Amino Butyric Acid) inhibitory local interneurones, with smooth dendrites. In each cortical layer, several pyramidal cell classes and many types of local interneurones contribute to selective local intra- and trans-laminar synaptic connectivity. The combination of functional as well as morphological and chemical neuronal diversity (McCormick et al., 1985; Markram et al., 2004) and selective connectivity (Thomson and Bannister, 2003; Perin et al., 2011) is expected to support the exquisite tuning of fast signal-propagation within the cortical network by differential dynamic filtering.

A large part of fast neuronal communication takes place chemically, via the release into the extracellular space of transmitter molecules by one cell, and the response of the recipient cell via specific interactions with receptor proteins in the plasma membrane. Fast neurotransmitter release occurs at specialised sites of contact between the pre- and post-synaptic cells called synapses (Foster and Sherrington, 1897). Typically for excitatory projections in the cortex, the presynaptic element would be an axon, contacting a postsynaptic dendrite. Synaptic connections between two neurones commonly involve more than one synapse.

The neocortex thus contains a large diversity of synaptic connection classes established with a great degree of selectivity. Hence, the rates at which connections between particular cell types can be detected, sometimes present discrepancies with estimates obtained from morphometrically derived synaptic densities alone (Binzegger et al., 2004; Thomson and Lamy, 2007). It appears, for example, that the evidenced connectivity between layer 2/3 pyramidal cells and large layer 5 pyramids, or between layer 4 spiny
excitatory cells and layer 2/3 pyramidal cells, does not reveal as many and potent recurrent connections. No such asymmetry is evident however, regarding inhibitory connections.

Many properties of synaptic connections have been shown to correlate with this connection selectivity. Excitatory connections onto layer 5 pyramidal cells for instance, commonly reveal to be stronger than those targeting pyramidal cells in layer 2/3 and layer 4. The pre- and post- synaptic cell types also correlate with the preponderance of synaptic dynamics expressed at some connections. For example, excitatory connections onto particular subtypes of inhibitory interneurones and/or from layer 6 cortico-thalamic pyramidal cells typically display a strong facilitating component, whereas depression dominates at most pyramid-pyramid connections. Properties of contacts made by a single axon also can differ in relation to the postsynaptic cell type. Different subtypes of interneurones are known to preferentially establish synaptic contacts over specific portions of their target cell neuritic processes (Markram et al., 2004; Douglas and Martin, 2009).

While much effort has been dedicated towards the detailed description of such properties for particular cortical connection classes, very few studies made it possible to integrate these properties further than one-to-one class comparisons.

This is why the studies presented in this thesis were designed to provide a functional picture of excitatory connections drawn at the scale of the neocortical microcircuit.
1.5 PROJECT AIMS

The project presented here aimed to study microcircuitry, specificity and properties of synaptic connections within the different layers of the adult rat neocortex.

Firstly, to study the frequency and potency of connections between the variety of neuronal types in each layer or across layers, in order to compare these with similar connections previously described in both mature and immature rat, and cat. To identify the pattern-dependent synaptic properties of excitatory connections, and to determine whether these properties are correlated with the morphological features and action potential discharge patterns of the connected cells.

Secondly, to establish whether some of the interrelations seen between determinants of the pattern-dependent synaptic properties can be replicated by a phenotypic model combining short term dynamics and release stochasticity.

Thirdly, to establish whether a methodological framework designed for obtaining a functional description of cortical excitatory connections through the simple binomial model of synaptic transmission is suitable in the case of connections with heterogeneous release properties, and if so, to which extent.

Lastly, to establish whether excitatory synaptic connections from the different neocortical layers differ in their functional profile, and propose corresponding estimates for the binomial parameters.
2.0 MATERIALS AND METHODS

2.1 SUMMARY OF THE TECHNIQUES

Dual intracellular recordings were performed in acute coronal slices of adult rat neocortex, coupled with histological marker (biocytin) filling of the recorded cells.

The electrophysiological characteristics of synaptically connected cells and the dynamic properties of the synaptic connections between them could therefore be put in relation to their respective anatomical shape and cortical laminar location after histochemical processing.

Computational models were developed to investigate the statistical outcomes of stochastic transmitter release based simulations of synaptic transmission, and to examine the relevance of methods designed for their experimental assessment.

2.2 ANIMALS AND ACUTE BRAIN SLICES

All procedures complied with British Home Office regulations for animal use.

2.2.1 Animals

Young adult male Wistar rats 85 - 210g were anaesthetised in an inhalation chamber (VetTech Solutions, UK) using Isoflurane (Abbott Laboratories Ltd., Queenborough, UK) saturated circulating medical Oxygen (BOC gases Medical). Once the righting reflexes ceased this initial anaesthesia was prolonged and deepened by intra-peritoneal injection of Euthatal (Merial, 60 mg/kg). Cessation of withdrawal reflex upon paw pinch indicated that the level of anaesthesia was suitable for surgery.
2.2.2 Surgery

The abdomen was opened by a large transverse incision into the peritoneum below the sternum. The base of the sternum was clamped using Spencer Wells forceps, then lifted up to cut the diaphragm. The ribs were cut on each side of the chest to expose the heart. A needle was then inserted into the left ventricle and 95% O₂ / 5% CO₂ (carbogen) equilibrated, ice cold sucrose artificial cerebrospinal fluid (ACSF) with Euthatal 100 mg.L⁻¹, was delivered by a peristaltic pump (Watson-Marlow 502s, Cornwall, UK).

Sucrose ACSF solution

<table>
<thead>
<tr>
<th>Chemical (AnalaR)</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Saccharose</td>
<td>248</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>25.5</td>
</tr>
<tr>
<td>KCl</td>
<td>3.3</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.2</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>1</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>2.5</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>15</td>
</tr>
</tbody>
</table>

The right atrium was cut to allow free flow of blood and perfusion medium from the body. Clearing of the flowing liquid indicated complete exsanguination at which point the animal was decapitated.

A medial sagittal incision of the scalp was performed to expose the skull and flat nodes pliers were used to remove the caudal bone plates, exposing the brain. Once the dura was removed a coronal scalpel cut was made through the frontal lobe. A spatula was inserted to scoop the resulting caudal portion of brain out of the skull and into a beaker containing fresh ice cold sucrose ACSF, whilst simultaneously severing the cranial nerves.
2.2.3 Slice preparation

The caudal portion of brain resulting from surgery was trimmed into a block containing the primary sensory regions of cortex (Bregma -7.0 to 0.5 mm; Rat Brain Brain Atlas, Paxinos & Watson, 4th Edition) which was glued onto the chuck of a Vibroslice (Campden Instruments, Loughborough, UK) using cyanoacrylate adhesive (Loctite, Super glue). Coronal sections 450-500 µm thick were cut serially and placed temporarily in a petri dish containing ice cold O₂/CO₂ equilibrated sucrose ACSF.

The slices were then transferred to the interface chamber where they were maintained in warm (34-35 °C) humidified O₂/CO₂ sucrose ACSF at a flow rate of approximately 0.5 ml.min⁻¹ supplied by a peristaltic pump (Gilson Minipuls 3, Wisconsin, USA) for one hour. The sucrose ACSF solution was then replaced with standard ACSF (see table on this page), all other conditions unchanged, for a further hour prior to electrophysiological recording.

### ACSF solution

<table>
<thead>
<tr>
<th>Chemical (AnalaR)</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
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</tr>
<tr>
<td>NaHCO₃</td>
<td>25.5</td>
</tr>
<tr>
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</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.2</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>1</td>
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<tr>
<td>CaCl₂</td>
<td>2.5</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>15</td>
</tr>
</tbody>
</table>
2.3 PAIRED INTRACELLULAR RECORDINGS

The wiring of the electrophysiology rig is summarised in figure 2.1.

2.3.2 The recording chamber and perfusion system

The recordings were performed in an interface holding chamber (figure 2.2) fitted above a Perpsex water jacket. The water jacket contained a heating pad and a thermocouple allowing respectively for the controlling and monitoring of its temperature. The design allowed the chamber to be provided with warm moist carbogen gas from below and aerated ACSF at 34-35 °C via a coil of the perfusion tubing inside the jacket.

2.3.3 Electrodes

Conventional sharp microelectrodes (resistance 90-200 MΩ) filled with a solution of 2% (w/v) biocytin in 2 M potassium methylsulfate were used for all paired intracellular recording experiments. These were produced from Borosilicate glass capillaries (Harvard apparatus, Edenbridge, UK) using a P-97 Flaming/Brown micropipette puller (Sutter Instruments, USA). The microelectrodes inserted in their holders were connected to the headstages (Axon Instruments, HS-2) mounted on micromanipulators (Prior, UK).

Sharp electrodes are more efficient for multiple recordings in thick slices of adult tissue which maintain the integrity of large neurones and local circuitry. The impaled cells were filled with biocytin (372.48 g.mol⁻¹, Molecular Weight) from the electrode, either by passive diffusion or by active iontophoresis using just threshold positive current pulses in a half duty cycle (1 Hz).
Figure 2.1: Schematic diagram summarising the wiring of the electrophysiology rig. The signals collected by the microelectrodes were amplified by the headstages first then by the amplifier, before being filtered by the signal conditioner. Three digital oscilloscopes were used to monitor neuronal activities in parallel, in both continuous real time and clock-triggered mode. The filtered analog signals were further converted to digital format by the signal digitizer to be recorded onto a personal computer. In-house script for the Spike2 software allowed the continuous visualisation of both channels whilst achieving the storage of the traces. White headed arrows depict the path of signals being recorded, black headed arrows for derived signals and commands.
Figure 2.2: Photographs illustrating the arrangement of the bath (A) and interface chamber (B) components of the electrophysiology rig. A: ACSF gets warmed and carbogen gas moistened using the temperature controlled water contained in the water jacket. B: The brain slices sit on a thin layer of tissue paper on the bottom of the chamber. The moistened carbogen gas is delivered to the interface chamber above via the jets located on 3 sides. The warmed equilibrated ACSF is delivered at the highest end of the inclined chamber. The circulation of gas and medium inside the chamber are optimised for the brain slice maintenance.
2.3.4 The amplifier

The AXOPROBE 1 amplifier (Axon Instruments, Burlingame, USA) consists of two independent multipurpose low-noise microelectrode amplifiers used for intracellular voltage recording and simultaneous current passing. In practice the amplifier was used to facilitate the impaling of neurones ('buzz' function), to display the differences in electric potential between the microelectrode and the reference electrode (ground) housed in the interface chamber (figure 2.2 B), to drive the membrane potential of impaled neurones through the passing of current and to monitor the measure in real time using a frequency modulated loudspeaker.

2.3.5 The oscilloscopes and timing device

The rig peripheral devices included a clock to set regular triggers for current injections at different rates (0.1, 0.33, 0.5, etc. Hz) and three digital oscilloscopes to monitor neuronal activity on two channels in parallel, both in continuous real time and clock-triggered modes. The triggered storage oscilloscopes thus allowed the visual inspection of the presynaptic cell response to synchronously triggered current injections and the simultaneous postsynaptic membrane potential in a sweep by sweep fashion. The time scale and voltage gain were adjusted so as to provide visual representation over the whole duration of the current injection on one oscilloscope and a close-up view on the second to allow synaptic events to be identified.
2.3.6 Signal filtering, digitising and recording

The signals of both channels of the AXOPROBE 1 amplifier were low-pass filtered at 5 kHz by a CED 1902 (Cambridge Electronic Design, UK) signal conditioner and converted from analog to digital format by a CED 1401 (Cambridge Electronic Design, UK) converter before being recorded onto a personal computer. A sample rate of 10 kHz (voltage resolution 0.005-0.01 mV) was chosen to produce a high resolution image of the original signal in accordance with the Nyquist Sampling Theorem. An in-house script for the Spike 2 (Cambridge Instruments Design, UK) software allowed the continuous visualisation of both channels being recorded and a choice of voltage scaling for each, whilst achieving the storage of the traces.

2.3.7 Targeting and search strategy

The first microelectrode was targeted at primary regions of visual or somatosensory cortex (excluding the barrel field) under visual control through the binocular microscope, and a neurone was impaled. Upon stabilization of this cell, provided passive and active membrane properties were deemed satisfactory, a second electrode was similarly inserted in close dorso-ventral ("columnar") alignment to the first in either an intra-, trans- or cross-laminar fashion depending on the connection class pursued. This second electrode was used to sample sequentially and test each impaled cell for synaptic connectivity with the first cell. A "test" consisted in eliciting action potentials (AP) in each cell and observing whether or not these evoked postsynaptic potentials (PSPs) in the other cell.

Neurones at several locations in the vicinity of the first insertion or at different points on the same columnar axis were tested. A map that kept track
of the insertions sanctioned by one or more impalements was constructed, until a synaptic connection was found.

The number of impaled cells within a search area was restricted to prevent compromising subsequent reconstruction efforts via fortuitous biocytin filling. A new search was then initiated in another cortical region, sufficiently far from the first search area to avoid excessive overlapping of filled neuronal processes.

If connectivity was observed, the cells were allowed to stabilise before AP were activated in the presynaptic cell. Positive square wave and/or ramp of current injections were delivered to the presynaptic one eliciting its firing of AP with an inter-trial interval of 3 seconds.

The characteristics of the presynaptic current injection were manually controlled and constantly adjusted, to obtain a range of presynaptic firing and immediate history of synaptic activity homogeneously distributed across the duration of the recording. Typically, the elicited trains included between 0 and 7 action potentials. The duration of the current pulse was between 150 and 500 milliseconds, typically 300 ms. The current intensity used was between 0 and 1000 pA.

Both pre and postsynaptic activities were then recorded using an in-house Spike2 script running on a personal computer. As the characteristics of postsynaptic responses are affected by spontaneous changes in membrane potential, the latter was monitored and controlled by manual current clamp. Additionally the postsynaptic electrode balance was monitored throughout the recording by applying a brief (<10 ms), small (<0.1 nA) negative current pulse prior to the presynaptic current pulse that elicited firing.
2.4 OFFLINE DATA MANAGEMENT

A second in-house Spike2 script was used to browse through the original data file offline and define time windows of standardised length for further analysis (termed “sweeps”). These were usually 300 ms in duration beginning 20-50 ms before the first presynaptic AP elicited by current injection. Sweeps that included large spontaneous events or artefacts were excluded from further analysis, and accepted sweeps stored in a data format compatible with in-house analysis software.

2.4.1 Electrophysiological analysis

In-house software designed to allow the simultaneous visualisation of pre- and post- synaptic electrophysiological waveforms on a sweep by sweep basis, was used for detailed analysis. Sweeps could then be further discarded or categorised according to a number of criteria including the quality of the recordings, the levels of spontaneous activity, the postsynaptic membrane potential and the properties of the neuronal responses to current pulse injections or spike-induced synaptic events.

2.4.1.1 Triggered averages

Trigger points aligned with the rising phase of each action potential were checked and where necessary manually adjusted. Averages of postsynaptic traces were generated using these triggers and the amplitude at the peak of the averaged PSP (voltage difference from baseline), its 10-90% rise time (RT, time required for the potential to rise from 10 to 90% of its peak amplitude) and width at half amplitude (HW, indicative of the envelope of the potential) were measured (figure 2.3 A).
Figure 2.3: Diagram illustrating the measurement of postsynaptic potentials. A: Single postsynaptic potential (PSP). The 10-90% rise time (RT) and width at half amplitude (half-width, HW) were derived from the measure of the average PSP amplitude between the peak and the baseline. B: Single sweep measurements of successive PSPs when the interval between presynaptic spikes was long enough for the preceding potential to decay fully back to baseline before the onset of the PSP being measured. C: Single sweep measurements of successive PSPs in the case of a shorter interval. The amplitude of the second PSP is taken from its peak to the corresponding point on the falling phase of the averaged single spike PSP scaled to match the preceding event.
Averages of PSPs activated by subsequent APs (2^{nd}, 3^{rd}, 4^{th}, etc. EPSPs) in a presynaptic train were also generated. For these averages, sweeps in which the interspike-interval (ISI) fell within a narrow time window (≤1ms) were selected. This allowed the construction of graphical representations of the time course of any changes in the characteristics of the successive PSPs as a function of ISI.

2.4.1.2 Single sweep measurements

The first PSP amplitude was measured as the difference between the peak of the PSP and the baseline measured prior to the presynaptic spike (figure 2.3). Subsequent PSPs in the same sweep could be measured in the same way, provided the preceding event was a transmission failure, or the preceding interspike interval was long enough to allow the preceding PSP to decay to baseline (figure 2.3 B). With shorter ISIs, the amplitude of the 2^{nd}, 3^{rd} or subsequent PSPs were measured as the difference between their peak voltage and the corresponding point on the decay of the averaged single spike PSP manually superimposed and scaled to match the preceding event (figure 2.3 C).

Failure of transmission events were identified when the postsynaptic trace did not present PSP characteristics following a presynaptic AP.

Single sweep data were plotted against interspike interval and smoothed (running average over 20 points or more) to reveal trends. This was achieved using the 1-D digital filter function called “filter” in MATLAB. The size of the smoothing window was selected as detailed for the example in section 3.2.2.2.

When enough single sweep measurements with a suitable range of interspike intervals for both second and third PSPs were collected, the
combined effects of successive interspike intervals and/or PSP amplitudes on the amplitude of the subsequent PSP amplitude could be rendered as colour coded three dimensional surface plots. These were obtained using the Delaunay triangle-based linear surface interpolation function “griddata” and the results plotted with the function “contourf” in MATLAB. The underlying interpolating grids were squares with sides 30, 40 or 60 subdivisions wide.

2.4.2 Fluctuation analysis

Single sweep measurements (section 2.4.1.2) were used to constitute subsets of data having similar conditions of transmission. The statistics of these subsets reflect functional properties of the connection (section 2.6.2).

To explore post-tetanic potentiation, first PSPs were sorted into subsets according to the number and frequency of spikes in the preceding spike train. This potentially led to the definition of multiple subsets for the first PSP in the train.

Data subsets for second and later PSPs were selected according to the interspike interval ranges over which the smoothed single sweep PSP amplitude was stable. An early selection could for example be achieved, of those interspike interval ranges where the values of the running first derivative of the later PSP amplitude against ISI, were continuously within one standard deviation of its average value. Only subsets comprising more than 20-30 points were selected.

The classical coefficient of variation method (CV^2; Faber and Korn, 1991) provided identification of pre- and post- synaptic loci involvement in the differences seen between subsets. In the simple binomial description, the mean amplitude (M) is the result of the product of the number of release
sites \( n \) times the average probability of release \( p \) times the quantal size \( q \):

\[
M = n \cdot p \cdot q
\]

The coefficient of variation \( CV \) of the amplitude on the other hand is independent of quantal size (McLachlan, 1978; also see section 2.6.2):

\[
CV = \frac{\sigma}{M} = \frac{\sqrt{\text{Var}}}{M} = \frac{\sqrt{np(1 - p)q^2}}{np} = \sqrt{\frac{np(1 - p)q^2}{n^2 p^2 q^2}} = \sqrt{\frac{1 - p}{np}}
\]

Therefore changes in synaptic efficacy between subsets can be described according to the relative changes affecting \( M \) and \( CV \). A whole range of situations may exist with, at both ends of the spectrum, presynaptically mediated changes appearing as changes in \( p \) which affect both \( M \) and \( CV \), whereas postsynaptic modifications associated with a change in \( q \) affect \( M \) while leaving \( CV \) unaffected.

Graphically this is reported as the ratio of \( CV^2 \) in the reference situation to \( CV^2 \) in the modified situation, as a function of the ratio of \( M \) in the modified situation to \( M \) in the reference:

\[
\frac{CV^2_{\text{ref}}}{CV^2_{\text{mod}}} = f\left(\frac{M_{\text{mod}}}{M_{\text{ref}}}\right)
\]

Thus, purely postsynaptic changes follow the horizontal line from the reference, mixed pre- and post- synaptic changes fall between the horizontal line and the identity line, and purely presynaptic changes pertain to the region between the identity line and the vertical line from the reference (Faber and Korn, 1991).
2.4.3 Fitting methods

To obtain estimates of the binomial parameters best describing each connection, nonlinear least square fittings were performed on the statistics of the subsets of PSPs selected as resulting from a presynaptically located change (i.e. change in release probability; section 2.4.1.3). The "Levenberg-Marquardt" algorithm with bisquare weights optimization method was used for the fitting procedure. When the optimization algorithm failed to converge, or convergence provided inconsistent sets of estimates using the 4 equations, the results obtained using different starting values were explored: close and halfway to higher bounds, close and halfway to lower bounds, and combinations of these. Typically this would not change the estimates provided, or the optima reached then showed lower values of the coefficient of determination ($r^2$).

The fitting used four different equations derived from the simple binomial model, based on the assumption that the subsets differed primarily in the probability of release, $p$ (standard binomial case illustrated in figure 2.4).

From $p = \frac{M}{nq}$ comes $Var = n.\frac{M}{nq}.\left(1 - \frac{M}{nq}\right).q^2$

That is $Var = qM - \frac{M^2}{n}$ Equation 1 (Sigworth, 1980)

Where $Var$ stands for variance and $M$ for mean amplitude. This equation describes a parabolic relation for the variance as a function of the mean amplitude of PSPs.
Figure 2.4: Plots of expected relations for CV (A), Failure rate (B), Variance (C) and Variance over Mean (D) against Mean Amplitude predicted in a simple binomial model of synaptic release, over a range of probabilities of release ($p$; 0.1 to 0.9). The green curves correspond to a situation common to all plots, i.e. number of release sites ($n$) of 7 and quantal size ($q$) of 0.5. The blue and red curves correspond to different values for $n$ (left; 4 and 10) and $q$ (right; 0.3 and 0.7).
A linearized version can be expressed:

\[
\frac{\text{Var} \ M}{M} = q - \frac{M}{n} \quad \text{Equation 2} \quad \text{(Scheuss and Neher, 2001)}
\]

This expression is useful in providing statistical tools to compare outcomes from different connections because it results in linear regressions for which these tools are available.

Similarly

\[
CV = \frac{\sqrt{\text{Var} \ M}}{M} = \frac{\sqrt{npq(1-p)}}{n} = \sqrt{\frac{(1-p)}{np}}
\]

Becomes

\[
CV = \sqrt{\frac{q - 1}{M \cdot np}} \quad \text{Equation 3}
\]

(Kuno, 1964; Silver et al., 1998)

Where \(CV\) stands for coefficient of variation. Equation 3 describes the relation of \(CV\) against \(M\) as the square root of an inverse function (figure 2.4 A).

These three equations are essentially reformulations of the same mathematical relation between the variability and average of PSP amplitudes in a range of release probability conditions.

A fourth relation considers specifically the PSP failure rate:

\[
\Pr(K = k) \quad \text{for} \quad k = 0
\]

Effectively

\[
F = \Pr(K = 0) = \frac{n!}{0!(n-0)!} \cdot p^0 \cdot (1-p)^{n-0} = (1-p)^n
\]
Again

\[ F = \left( 1 - \frac{M}{nq} \right)^n \]  

Equation 4

Where \( F \) stands for failure rate, i.e. the ratio of the number of events showing an absence of release over the total number of events. This relation as extracted from the definition of the binomial expansion has, importantly, one measure – failure rate – that is independent of the quantal size (\( q \)).

Allowing an assessment that is independent of equations 1-3, it is, however, less reliable in conditions of high release probability and/or numerous release sites because of the resultant scarcity of failure events, which leads to poor estimates of \( F \).

By identifying the curves obeying equations 1-4 that best fit the data from subsets of PSPs, least square algorithms provide the values of \( n \) and \( q \) and by extrapolation, \( p \), that best describe the functional profile of a connection.

2.4.4 Data considerations

In figures derived from experimental traces, capacity coupling artefacts in the postsynaptic trace corresponding to the onset and end of presynaptic current injection and to presynaptic spike were removed graphically.

All connections in this study were recorded in the absence of blockade of GABA receptors. This classically leaves the possibility of recruitment or spontaneous firing of interneurones, potentially complicating the interpretation of the evoked EPSPs. Although the possibility of inhibitory inputs influencing locally the propagation of excitatory inputs in distant part of
the dendritic tree cannot be ruled out, only in very few occasions was a consistent inhibitory input detected at the cell body in any of these paired recordings. Furthermore, sweeps in which EPSP shape could be suspected to be altered were systematically excluded from any analysis. All conclusions drawn here should therefore hold true within the context of local inhibitory influence.

Numbers given in the text are mean ± standard deviation, unless otherwise stated.
2.5 HISTOLOGICAL PROCEDURE

2.5.1 Fixation

After electrophysiological recordings and biocytin filling, the microelectrodes were carefully withdrawn from the slice. The slices were trimmed while in the interface chamber to isolate the portion of tissue that contained the recorded neurones. This portion of slice was placed between two pieces of micropore filter paper, moistened with an aldehyde containing fixative solution, to keep the tissue flat. This assembly was then submerged in the fixative solution and stored overnight at 4°C, then washed with 0.1 M Phosphate Buffer (PB).

2.5.2 Sectioning

Sections 50-60 μm thick were then cut from the 450-500 μm thick slices. Briefly, the slices were embedded in a warm 12% aqueous gelatine solution. This was then cooled at 4°C and the gelatine hardened in the aldehydic fixative solution for one hour. A block of gelatine containing the tissue was glued onto the stage of a Vibratome (Agar Scientific, Stanstead, UK) using cyanoacrylate adhesive (Loctite Superglue) and 50-60 μm thick coronal sections cut in cold 0.1 M PB. All sections were sketched and counted carefully as they were cut then transferred to a glass vial containing fresh 0.1 M PB.
2.5.3 Tissue processing

The gelatine was trimmed from the sections using a scalpel, under a stereoscopic dissecting microscope. All subsequent incubations were performed on free-floating sections in the glass vial subjected to constant gentle agitation on a Titramax 100 rotary shaker (Heidolph Instruments, Schwabach, Germany).

2.5.4 Permeabilisation

The penetration of large molecules, such as antibodies and horse radish peroxidase (HRP), to the cell interior requires permeabilisation of cell membranes. This was achieved with the rapid formation of ice crystals induced by a freeze/thaw process. Sections were cryoprotected in graded 0.1M PB based solutions containing 10, 20 then 30% (w/v) sucrose and respectively 0, 6 then 12% glycerol, then placed flat into aluminium foil blotted dry, and the foil folded into a "parcel". Repeated freeze and thaw cycles involved transferring this parcel between the surface of liquid nitrogen and room temperature (3 times), before returning the sections to 0.1M PB.

2.5.5 Biocytin visualisation

The visualisation of biocytin injected into recorded neurones relies upon the Avidin-Biotin-HRP Complex (ABC peroxidase) technique. This protocol utilises the binding of biocytin by an Avidin/Horse Radish Peroxidase (HRP) complex. The HRP is then used to catalyse the reduction of H$_2$O$_2$ to facilitate the oxidation of 3,3 Diaminobenzidine tetrahydrochloride (DAB), generating a permanent and coloured pigment, discretely localized at the HRP-labeled sites (figure 2.5), and visible at the light microscope.
**Figure 2.5: Schematic illustrating reagent interactions for the permanent biocytin visualisation.** The visualisation of the biocytin (B) injected within the recorded neurones relies upon the Avidin-Biotin-HRP Complex (ABC peroxidase) technique. This protocol utilises the binding of biocytin by an Avidin/Horse Radish Peroxidase (HRP) complex (respectively red and green). The HRP is then used to catalyse the reduction of $H_2O_2$ to facilitate the oxidation of 3,3 Diaminobenzidine tetrahydrochloride (DAB), generating a coloured pigment (stars) discretely localized at the HRP-labeled sites, hence visible with light microscopy.
The cellular tracer biocytin used to fill the cells is a derivative of the soluble vitamin biotin (also commonly referred to as Vitamin H or B7). It has a low molecular weight of 372.48 Daltons and a high, effectively irreversible, affinity for the glycoprotein Avidin. The egg white derived protein Avidin (MW 68 kDa) comprises four identical subunits each capable of binding biocytin. In the ABC peroxidase solution (Vector Laboratories, Peterborough, UK), one of the biotin binding sites is occupied by a molecule of biotynilated HRP. HRP (MW 40 kDa) is the detection molecule, its enzymatic activity catalyses the breakdown of H₂O₂ (0.01 % for optimal stoichiometry) into H₂O. The oxygen liberated by this reaction is then available to oxidise the substrate DAB which polymerises forming a non-soluble, local, stable, brown/black coloured precipitate. The staining can be further intensified to dark blue/dark in the presence of nickel or cobalt chloride.

Permeabilised sections were incubated in a solution of ABC peroxidase in 0.1 M Phosphate Buffer Saline (PBS) overnight at 4 °C on a shaker. After washing, the sections were incubated for 15 minutes in a solution of DAB (Sigma-Aldrich, St Louis, USA) in PBS containing one drop of 8 % nickel chloride (NiCl₂), prior to the peroxidase reaction initiated by the application of 1 % hydrogen peroxide. The reaction was allowed to proceed until positively stained neurones were clearly visible under the dissecting microscope.

2.5.6 Post fixation

Following the staining procedure, the sections were subjected to post fixation using osmium tetroxide to fix cell membranes prior to dehydration and to enhance contrast for microscopy. Sections were placed flat between
two pieces of filter paper. This assembly was impregnated with 1% osmium tetroxide in 0.1M PB for 30 minutes in a fume hood.

2.5.7 Dehydration

As the epoxy resin used to embed tissue sections is not miscible with water, it was necessary to dehydrate the tissue through graded ethanol solutions. The sections were placed on a clean microscope slide and held flat by a coverglass. Graded ethanol solutions (50, 70, 95 and 100%) were applied sequentially, each for a minimum of 15 minutes with gentle agitation to ensure optimal dehydration minimising shrinkage artefacts and conserving shape.

2.5.8 Clearing, Embedding and Curing

The alcohol was replaced by propylene oxide (C₃H₆O, 2x 10 minutes) followed by ducurpan epoxy resin (Fluka, Steinheim, Switzerland) mixed in propylene oxide (1:1) for 30 minutes. The sections were then transferred to a small aluminium planchette containing undiluted unpolymerised epoxy resin for a minimum of three hours in a fume hood to allow for the evaporation of the linking agent and infiltration of resin into the tissue spaces.

The sections were then mounted onto glass slides, coverslipped and cured at 56°C for 48 hours. At this point the mounted slides can be stored indefinitely.
2.6 MODELING STUDIES

2.6.1 Binomial theory

In probability theory and statistics, the binomial distribution is the discrete probability distribution of the number of successes in a sequence of \( n \) independent draws having a binary individual outcome: success with probability \( p \) or failure with probability \( 1 - p \).

Let \( K \) be a random variable following the binomial distribution with parameters \( n \) and \( p \):

\[
K \sim B(n, p)
\]

The probability of getting exactly \( k \) successes out of \( n \) draws is given by the following probability mass function:

\[
f(n,p;k) = C(n,k) \cdot p^k \cdot (1 - p)^{n-k} \quad \text{for} \quad k = 0, 1, 2, ..., n
\]

Where \( C(n,k) \) is the binomial coefficient (number of arrangements of \( k \) successes along \( n \) draws):

\[
C(n,k) = \frac{n!}{k!(n-k)!}
\]

2.6.2 Binomial description of transmitter release statistics

Following Bennett and Kearns (2000) in their formulation of the approach introduced by del Castillo and Katz (1954), we suppose that a synaptic connection can be described as made of \( n \) identical release units with an average probability of responding \( p \).
We can define the random variable $X_i$ for $i = 1, 2, \ldots, n$ such that $X_i = 0$ if the $i^{th}$ unit does not respond ("failure") and $X_i = 1$ if it does respond ("success").

Then $P(X_i = 0) = 1 - p$

And $P(X_i = 1) = p$ for $i = 1, 2, \ldots, n$

Importantly, we here need to assume that the $n$ units respond independently of one another so that the $X_i$s are mutually stochastically independent (exactly like successive draws from a single unit).

Then the sum $K = X_1 + X_2 + \ldots + X_n$ is the number of units responding over one trial, and $K$ is a random variable following the binomial distribution.

Therefore,

$$\Pr(K = k) = \binom{n}{k} p^k (1 - p)^{n-k} \text{ for } k = 0, 1, 2, \ldots, n$$

which is the probability density function (pdf) of the binomial distribution.

Using the moment generating function to determine the mean $\mu_k$ and variance $\sigma^2_k$:

$$M(t) = E(e^{tK}) = [(1 - p) + pe^t]^n$$

Then

$$M'(t) = n[(1 - p) + pe^t]^{n-1}(pe^t)$$

$$M''(t) = n(n-1)[(1 - p) + pe^t]^{n-2}(pe^t)^2 + n[(1 - p) + pe^t]^{n-1}(pe^t)$$

Hence

$$M'(0) = np$$
\[ M''(0) = n(n - 1)p^2 + np \]

Therefore,
\[ \mu k = np \quad \text{and} \quad \sigma^2_k = np(1 - p) \]

Restricting the model to comply with the "quantal hypothesis" for transmitter release, requires that unitary responses, being uniform, can be considered identical and amounting each to a single quantum \((q)\):

\[ M = npq \quad \text{and} \quad Var = np(1 - p)q^2 \]

(Sigworth, 1980; Silver, 2003)

Where \(M\) and \(Var\) stand for mean and variance respectively. Estimates of the parameters may thus be sought by calculating the corresponding statistics from a sample of responses.

### 2.6.3 Simulated binomial models

More complex binomial models were designed to challenge the fitting methods used on experimental data and based on the simple binomial model (see section 2.4.1.4), with the statistics from the subsets of amplitudes that they would generate.

These models had the following parameters: number of release sites \((n)\), individual quantal size \((q)\), individual probability of release \((p)\) (individual \(q\) and \(p\) would be identical at all sites in the simple binomial model), and correlation status between \(q\) and \(p\) across sites (uncorrelated, correlated or anti-correlated).

A range of probability of release conditions was required to allow the fitting methods to be used on the generated subsets. Initial average \(p\).
values of 0.2 (low $p$-conditions) or 0.8 (high $p$-conditions) were respectively increased or decreased by proportional steps to define the different $p$-conditions. The individual $p$ and $q$ values of the release sites were then distributed regularly around the corresponding mean value in the simple binomial model (specimen in table 2.1).

Six models were analysed:

**Simple binomial model:** $p$ and $q$ identical within a probability condition (wpc).

**Complex $q$ changing:** $p$ identical but $q$ varied across sites wpc.

**Complex $p$ changing:** $q$ identical but $p$ varied across sites wpc.

**Complex $p$&$q$ uncorrelated:** $p$ and $q$ varied across sites wpc, with no correlation.

**Complex $p$&$q$ correlated:** Same as above but $p$ and $q$ values positively correlated across sites wpc; the sites with bigger $p$ have bigger $q$.

**Complex $p$&$q$ anti-correlated:** Same as above but $p$ and $q$ values negatively correlated across sites wpc; the sites with bigger $p$ have smaller $q$.

One draw was then the process of:

- Generating as many random numbers uniformly distributed between 0 and 1 as there were release sites.
- Comparing these to the specific probability of release of their respective release site as a threshold for the all-or-none (binary) individual outcome.
- Setting each outcome to the specific quantal size of the release site in the case of success, or to zero in the case of failure.
- Summing up to get the amplitude of the resulting simulated event.
<table>
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<th>Binomial model</th>
<th>Site #</th>
<th>Initial p-condition</th>
<th>Step</th>
<th>Second p-condition</th>
<th>Final (10&lt;sup&gt;th&lt;/sup&gt;) p-condition</th>
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<td></td>
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<td>individual p</td>
<td>f</td>
<td>individual p</td>
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</table>

Table 2.1: Individual site parameters for Monte-Carlo simulation of a complex binomial model in which values for p and q at each site are correlated. In the simple binomial model all release sites have identical probability of release (p) and quantal size (q). Here instead, the release sites have individual values of p (individual p) and q (individual q). This instance of the model considers a connection made of 8 release sites (Site#). Individual q and p values are generated as regularly spaced over an interval around the mean value in the corresponding simple binomial model (e.g. in the last row: 1.00±0.43 and 0.8±0.13 for q and p respectively). These values are assigned to each site according to the relation between p and q across sites, i.e. whether these two parameters were correlated (presented here), anti-correlated or uncorrelated. 10 different p-conditions were defined with individual p incremented by a proportional amount (Step) each time from the initial p-condition. Two possibilities are detailed: low (penultimate row) and high (last row) mean p-conditions. These individual p and q values are used in Monte-Carlo simulations of this particular binomial model.
2.6.4 Monte Carlo simulations

For each of the 6 models, 500 simulations were performed for statistical assessment of the performance of the fitting methods.

Each simulation generated samples of 60 (experimental like) or 300 (for variability convergence) draws according to the set of probability of release conditions to be explored.

The outcomes from such simulations allowed the relations between mean amplitude and measures of variability in the different binomial models over the range of p-conditions considered to be represented, and their differences relative to the simple binomial case to be assessed.

In a second step, the 4 fitting methods were performed on the values of mean and measures of variability from the samples in each of the 500 simulations.

Each fit performed, generated an estimated value for n and mean q that best described the functional profile in a simple binomial model paradigm, as well as the goodness-of-fit measure R-square. These data were collected together with the corresponding calculated estimates for initial mean probability of release.

The cumulative distribution of quantal parameter estimates in the simple binomial model case (figure 3.16) served as a control: it had a sigmoid shape reflecting the underlying Gaussian distribution, and reached half accumulation (median value) exactly at original parameter values (n=8 or q=1.00). This absence of deviation in the parameters estimated validated the suitability of the random number generators used for Monte-Carlo simulations.
2.6.5 Statistical assessment

The distributions of estimates obtained from different models all with the same mean probability of release conditions were compared for differences in mean values by performing Student's $t$-test at significance levels 0.01 and 0.05. Because some distributions were either not presenting a Gaussian profile or not presenting the same spread of values, all were also compared by two-sample Kolmogorov-Smirnov test (non parametric test of common continuous distribution) at significance level 0.01 and 0.05.

This allowed the ability of the fitting methods in estimating binomial parameters from statistics generated by the different release models to be compared.

2.6.6 Dynamic stochastic model

The dynamic stochastic model of synaptic transmission was modified from the phenomenological model for activity dependent properties of synaptic transmission described in Tsodyks et al. (1998). The original model considered two variables, the "synaptic resource" and a "facilitation variable" undergoing time dependent recovery cycles following recruitment by a presynaptic action potential. Both variables work as fractions of the corresponding synaptic characteristics, allowing for intrinsic boundaries in the model behaviour.

The model was implemented and simulated using NEURON 6.1 (Hines and Carnevale, 1997).

The modifications made to the original model were intended to allow the simulation of the dynamics of each release site independently of each other, as well as the implementation of a stochastic nature in the transmission process that could be affected by these release site dynamics (figure 2.6).
The postynaptic side of the model consisted of a single cylindrical compartment with axial resistivity $Ra = 100$ (Ohm.cm) and specific capacitance $Cm = 1$ (μF.cm$^2$). Passive conductances were inserted with the following characteristics: conductance $g_{\text{pas}} = 1 \times 10^{-3}$ (S.cm$^2$) and reversal potential $E_{\text{pas}} = -72$ (mV). Voltage dependent conductances that allowed realistic action potential (AP) generation at elevated temperature—the temperature was set at $celsius = 35$ ($^\circ$C) — were modelled using the $hh2$ mechanism (Destexhe et al., 1998) with the following parameters:

- potassium conductances, $g_{Khh2} = 5 \times 10^{-3}$ (S.cm$^2$)
- sodium conductances, $g_{Nahh2} = 9 \times 10^{-2}$ (S.cm$^2$)
- threshold adjusting variable, $V_{\text{traub\_hh2}} = -63$ (mV).

As many instances of the mechanism governing release site behaviour were inserted as there were release sites to be simulated. The articulation between presynaptic triggers and postsynaptic activity using the release site mechanisms was managed by the ad hoc NEURON class NetCon.

The simulations consisted of 500 ms epochs (sweeps) having identical initial conditions. Presynaptic triggers were restricted to a 300 ms period following an initial 100 ms delay, long enough for postsynaptic membrane potential to reach resting level.

At the idealized connections considered, the probability of release at each release site was recovered dynamically from levels decreased upon transmission. In this configuration of the model, presynaptic triggers elicited a quantal transmission at every site where the outcome of an individual random number generator (uniform between 0 and 1; modified from Carnevale T.'s
Binexp.mod – http://www.neuron.yale.edu/ftp/ted/neuron/ chance.zip, stochastic part) did not exceed the current probability of release \( (p, \text{ initial } p_0) \). Immediately after the trigger, \( p \) was set to \( p/2 \). Similarly to the resource variable in the original model, the remaining quantity \( p/2 \) was (also immediately) transferred to an "inactive state" variable \( (y) \) consequently set to \( y + p/2 \). The recovery cycle for the variable \( p \) from this "inactive state" included a third state variable \( (z) \) – analogous to the "recovered" state in the original model. Thus during recovery, \( y \) decreased exponentially to zero, transferring into \( z \) that itself was decreasing back to zero progressively reestablishing \( p \) to its initial level \( p_0 \). Recoveries from inactivated and from recovered states happened with specific time constants respectively \( \tau_{\text{in}} \) and \( \tau_{\text{rec}} \).

The corresponding set of kinetic equations (schematised in figure 2.6) reads:

\[
\frac{dy}{dt} = -\frac{y}{\tau_{\text{in}}}
\]

\[
\frac{dz}{dt} = \frac{y}{\tau_{\text{in}}} - \frac{z}{\tau_{\text{rec}}}
\]

\[
p = p_0 (1 - y - z)
\]

The postsynaptic current \( (i) \) induced at each site consisted of a sharp increase in conductance \( (g) \) (except in case of a transmission failure), multiplied by the driving force generated by the difference between the current postsynaptic membrane potential \( (V) \) and the equilibrium potential of this unspecific current \( (e) \) chosen to be 0:
Figure 2.6: Diagram illustrating the mechanisms linking stochastic release and its dynamics at each release site in the dynamic stochastic model. Upon the presynaptic trigger (red, stylised as an action potential), the current value of release probability ($p$) is challenged with the outcome of a random number generator uniform between 0 and 1. Failure to release (internal dotted red circle) ensues if this number is greater than $p$. Success, i.e. release (external dotted red circle) ensues if the number is smaller than $p$. This triggers ($k=1$) the postsynaptic conductance ($g$) which supports the generation of local depolarisation (orange, rising phase of the stylised PSP) according to the established driving force. The conductance thereafter decays back exponentially to 0 ($\tau_1$), while $p$ follows a two state recovery process (top black arrows) with time constants $\tau_{in}$ and $\tau_{rec}$. 
\[ i = g_e (e - V) \]

The conductance thereafter, in the absence of another presynaptic trigger, was set to decay exponentially with time back to 0.

\[ \frac{dg}{dt} = -\frac{g}{\tau_1} \]

The amplitudes of successive simulated postsynaptic depolarisations (figure 2.7) were measured automatically using a fitting procedure that reproduced the steps taken during the analysis performed manually on experimental recordings. Namely, the prototypical shape of a dummy run forcing release at a single site was fitted with a double exponential equation using a least mean square fitting method. Thereafter, the voltage trace obtained from each simulation run was divided into sequences based on presynaptic trigger times. The peak voltage of the EPSP was extracted and the prototypical shape adjusted to the shape of the preceding event. Thus, upon measurement of voltage at peak it was possible to extrapolate the corresponding point on a putative decay from the preceding depolarisation (see figure 2.3 C). The amplitude measured was then the difference between the voltage at the peak of the EPSP and the voltage reached along the putative decay of the preceding depolarisation.
Figure 2.7: Screen captures of the dynamic stochastic model simulation interface.  
A: The NEURON environment provides a RunControl standard panel for the simulation controls. Here, the parameters in use were: initial membrane potential (Init), amount of time to be simulated (Tstop) and simulation time step (dt). B: The Dynamic Stochastic controls panel was designed to reference individual release site characteristics from the model. Parameters determining the dynamics of probability of release and quantal size (respectively primed by the letter 'p' or 'q') are listed on the left, those defining the stochastic properties on the right for four different sites in this example. In this case quantal size is constant as $q_{tau_1} \geq q_{tau_{rec}}$. STO, switch on/off stochasticity. $p_0$, initial probability of release. $p_f$, switch between dynamical schemes for $p$. $p_h$, switch on/off individual site dynamics. $x_0$, initial fraction of quantal size, adjustment variable. $q_f$ and $q_h$ are equivalent to $p_f$ and $p_h$ but applying to quantal size. C: Graphical display of 4 simulation runs with identical specifications (see A and B) stressing the stochastic nature of transmission in the model. Six presynaptic triggers for release were set every 50ms starting after 100ms. Traces represent membrane potential (in mV) as a function of time (in ms), colour depicting the distinct simulation runs. Depending on how many sites are successful in eliciting quantal release upon each presynaptic trigger, the completeness of preceding potential decay and postsynaptic driving force, the amplitude of the resulting summated potential varies. These amplitudes were measured through an automated fitting procedure based on the rescaling of a standard single potential to match each waveform allowing to infer the potential reached along the decay of the preceding potential when needed.
3.0 RESULTS

3.1 OVERVIEW OF EXPERIMENTS

3.1.1 General properties of tested neurones

In the course of this study, one hundred and twelve experiments were performed in primary sensory areas of slices of adult rat neocortex. In total, intracellular monitoring of 4484 cells was established, enabling the assessment of 7120 potential synaptic connections indicated by postsynaptic potentials (PSPs) in response to evoked presynaptic action potentials. In total, 924 neurones were impaled with the first sharp microelectrode, held and tested for connections with 3560 other neurones impaled with a second sharp microelectrode.

Of the 924 neurones impaled and held with the first electrode, 917 were electrophysiologically classified according to the passive electrical properties of their membrane, action potential shape/duration, intrinsic firing pattern (figure 3.1) and the PSPs they generated in coupled cells. 849 were predicted by their intrinsic properties, or confirmed by the excitatory postsynaptic potentials (EPSPs) they generated, to be excitatory cells in layers 2 to 6 of the neocortex. Of these 849, 734 displayed adapting characteristics, inclusive of strongly adapting cortico-cortical (CC) cells in layer 6 and cells in which only a single spike was elicited (21 in layer 2, 447 in layer 3, 109 in layer 4, 50 in layer 5, 67 in layer 6 and 40 in unconfirmed layers). 112 cells displayed non-adapting patterns, inclusive of intrinsic burst firing (iBF) mostly in layer 5, sustained adapting of corticothalamic (CT) cells in layer 6 and burst-adapting ("bad"); short bursts of 3-5 APs at the onset of the depolarizing current pulse, followed by single spikes with progressively increasing interspike intervals) in the other layers (1 in layer 2, 15 in layer 3,
Figure 3.1: Representative firing patterns in the neocortex. Passive (averages in black) and active (single sweep traces in color) electrophysiological responses of a diversity of recorded cells (A - F) to negative and positive current pulses (illustrated in A) are represented. A1: adapting firing pattern of a pyramidal cell in layer 4, the frequency of firing decreases upon the steady application of current. A2: intrinsic bursting pattern of a layer 5 pyramidal cell, features an initial period of firing followed by sustained adaptation that may subsequently (not represented) give place to either similar bursts-adaptation sequences or adapted firing. B1: strongly adapting pattern characteristic of a cortico-cortical cell in layer 6. B2: long lasting adaptation characteristic of a cortico-thalamic cell in layer 6. C1: adapting layer 3 pyramidal cell. C2: Non adapating, interrupting firing pattern with deep fast afterhyperpolarization typical of an interneurone.
22 in layer 4, 26 in layer 5, 30 in layer 6, and 18 in unconfirmed layers). In the 3 remaining cases the cells being held and tested were displaying dendritic electrophysiological characteristics.

Sixty seven electrophysiologically characterised neurones were predicted by their membrane passive electrical properties and discharge pattern, or confirmed by the inhibitory postsynaptic potentials (IPSPs) they generated to be inhibitory cells in layers 3 to 6 (28 in layer 6, 8 in layer 4, 2 in layer 5, 27 in layer 6 and 2 in unconfirmed layers).

3.1.2 Connections and hit rates

The simultaneous recording of pairs of neurones and testing for synaptic connections in both directions allowed the level of connectivity (hit rate) between broadly classified neurones in different layers of the rat neocortex to be estimated (see section 2.3.7).

From a total of 3560 pairs of neurones thus tested, 72 were synaptically connected, providing an average hit rate over this experiment series of one connection every 99 tests. Of these, 67 were excitatory connections of which 57 were to other excitatory cells (3 reciprocally) and 10 were to inhibitory cells. 5 inhibitory connections were found onto excitatory cells. The hit rates for positively identified synaptically connected pairs are summarised in figure 3.2.

3.1.3 Connection class properties

When suitably long and stable recordings could be obtained from a pair of connected neurones, the electrophysiological properties of the postsynaptic response to a single presynaptic action potential were determined (see figure 2.3 and section 2.4.1.2). The measurements for the
Figure 3.2: Summary diagram of the cortical connectivity identified in the course of this study. The diagram is organised as a coronal view of the neocortex, with the dotted lines representing the boundaries between the layers from layer 2 (top) to layer 6 (bottom). The pyramidal cells are depicted by triangles and subdivided in two groups according to the strictly adapting or otherwise (burst-adapting in layer 2-4, intrinsic bursting in L5, and sustained adapting in L6) nature of their firing pattern, while the interneurons are depicted by circles as a single class. Single headed arrows indicate identified connections between cell types, while double headed arrows indicate reciprocal connections. Hit rates are indicated for these connection classes for which a sufficient sample was collected.
individual connections constituting the different classes are detailed in table 3.1, together with the results from the statistical tests performed (t-test columns in table 3.1) and illustrated in figure 3.3.

No differences between connection classes were seen regarding the membrane potential at which the postsynaptic cells were recorded (figure 3.3 A, mb. Pot. column in table 3.1).

In contrast, as shown in figure 3.3 B, excitatory post-synaptic potentials (EPSPs) in layer 5 (L5) appeared to display, on average, a larger mean amplitude (Ampl. column in table 3.1) than those in layers 3 and 6 (L3a and L6 respectively). Within layer 3, the mean amplitude of EPSPs for connections in which the postsynaptic cell displayed an adapting firing pattern (L3a) was also indicated to be smaller than for those onto burst-adapting cells (L3b).

Connections in different layers also appeared to differ in the width at half amplitude of their average EPSP (HW column in table 3.1, and figure 3.3 C). In layer 3, it was found to be longer on average for connections onto adapting cells (L3a) than for connections in which the postsynaptic cell displayed an interneuronal firing pattern (L3i). These layer 3 EPSPs with a long half width were also indicated to be broader than layer 6 EPSPs. The layer 6 connections also displayed, on average, a shorter EPSP half-width than connections in layer 5.

No difference in the 10-90% rise time (RT) could be seen between these connection classes in the different cortical layers.

The differences in width at half amplitude and rise time between connections in which the postsynaptic cell displayed an adapting or burst-adapting firing pattern within layer 3 (L3a and L3b respectively) were not apparent in layer 4 where the number of recordings from each class (L4a and
L4b respectively) allowed the same intralaminar comparisons to be performed (table 3.1). Therefore a single class of layer 4 excitatory connections (L4) was considered for the comparisons of EPSP characteristics to connection classes in other layers. Similarly a single connection class was considered in layer 5 (L5) and one in layer 6 (L6).
Table 3.1: Electrophysiological properties of classes of excitatory connections in different neocortical layers. From left to right, columns indicate, the cortical layer and connection class considered (Layer), a reference to the experiment during which the data were collected (Experiment), the firing patterns of the presynaptic (Presyn.) and postsynaptic (Postsyn.) cells, the excitatory or inhibitory nature of the event recorded (Event), the postsynaptic membrane potential at which the recordings were performed (mb. Pot.), the peak amplitude of the event (Ampl.), classes that differed in their Ampl. values (f-test Ampl.), the width at half peak amplitude of the average event (HW), the corresponding statistical outcome (f-test HW), the rise time from 10 to 90% of peak amplitude of the average event (RT) and the corresponding statistical outcome (f-test RT). Statistical assessment from Student's t-test with Bonferroni correction for multiple comparisons at significance level 0.1 and 0.05 (bold) [for visualisation see figure 3.3].
Figure 3.3: Electrophysiological properties of classes of excitatory connections in different neocortical layers. The postsynaptic membrane potential (A), peak amplitude of the average excitatory postsynaptic potential (EPSP, B), width at half amplitude (C) and rise time from 10 to 90% of peak amplitude (D) from different cortical connection classes are shown. Average values are depicted with error bars representing the standard deviation. The number of connections in each class appears above the x-axis of figure B. Connection classes: L3a, layer 3 with postsynaptic cell adapting; L3b, layer 3 with postsynaptic cell burst-adapting; L3i, layer 3 with postsynaptic interneurone; L4a, layer 4 with postsynaptic cell adapting; L4b, layer 4 with postsynaptic cell burst-adapting; L4, merging L4a and L4b classes; L5, connections in layer 5; L6, connections in layer 6. Statistical assessment from Student t-test with Bonferroni correction for multiple comparisons, at significance level 0.1 (*), 0.05 (**).
3.2 SYNAPTIC PROPERTIES OF NEOCORTICAL CONNECTIONS

Paired electrophysiological recordings with biocytin filling were performed to investigate synaptic connectivity in the different layers of the adult rat neocortex. Visualisation of the recorded cells was used to confirm electrophysiological cues about their respective laminar location and morphological features. Analysis of electrophysiological recordings was performed to establish the EPSP properties including paired pulse and frequency dependent properties employed at their synaptic connections.

3.2.1 Synaptic dynamics

The stability of postsynaptic electrophysiological properties was monitored throughout the recordings and evaluated in the first steps of the analysis. These included the resting membrane potential, resistance, capacitance and single PSP characteristics (amplitude and standard deviation time-course). Further analysis of recorded EPSPs was performed on stable data sets.

3.2.1.1 Short term depression

Results obtained from a typical connection in cortical layer 5 are illustrated in figure 3.4. The postsynaptic cell was a large pyramidal cell shown in the photomicrograph (figure 3.4 A). This cell generated broadening action potentials during an initial burst which was followed by adaptive firing (insert in figure 3.4 B). The average waveform of the EPSPs elicited by single presynaptic action potentials is represented in figure 3.4 B. The peak amplitude for this average response was 1.80 mV, its rise time from 10 to 90 % of peak amplitude was 2.1 ms and the width at half amplitude was
Figure 3.4: Electrophysiological characteristics of an excitatory connection onto a large burst firing pyramidal cell in layer 5 of the neocortex. A: photomicrograph of the postsynaptic cell filled with biocytin as revealed by HRP reaction. B: The average trace (yellow) of EPSPs elicited in response to a single presynaptic action potential (black) is represented (amplitude, 1.80 mV; width at half amplitude, 11.7 ms; rise time, 2.10 ms). Insert, top right, shows responses (black) and firing pattern (yellow) of the postsynaptic cell to current injections. C: At short interspike intervals, the average response to the second action potential displayed a smaller amplitude than the first.
11.7 ms. When more than one action potential was triggered presynaptically, the peak amplitude for the average EPSP triggered by the second action potentials was smaller than the peak amplitude of the first EPSP (figure 3.4 C), i.e. this connection, like many pyramid-pyramid connections, displayed depression.

3.2.1.2 Short term facilitation

The electrophysiological characteristics of a connection from a putative corticothalamic (CT) cell in layer 6 to a corticocortical (CC) pyramid are illustrated in figure 3.5. The average EPSP elicited by the first presynaptic spike is illustrated in black, with the last part of its decay phase represented as a dotted line (figure 3.5 A). The peak amplitude for this average response was 0.48 mV, its rise time from 10 to 90 % of peak amplitude was 5.80 ms and width at half amplitude was 0.40 ms. The average EPSP triggered by the second presynaptic action potential over a range of interspike intervals are colour coded according to their respective peak amplitude. At the shortest interspike intervals recorded, the second EPSP displayed values of peak amplitude dramatically increased compared with the first EPSP (maximum of 1.37 mV, a 2.8 fold increase in amplitude). This EPSP, like other CT cell outputs, but unlike EPSPs generated by layer 6 CC cells, or indeed by most pyramid-pyramid connections in other layers, displayed facilitation.

As the interspike intervals increased the facilitation declined. This trend is represented in figure 3.5 B, where peak amplitudes are plotted against the preceding interspike interval for the 2nd and 3rd EPSPs in brief trains. The apparent rate of recovery from facilitation was estimated by fitting the data with single exponentials. The recovery from facilitation of the 2nd EPSP
Figure 3.5: Cortical connection displaying facilitation of the postsynaptic response. A: Over a range of interspike intervals this EPSP from a layer 6 corticothalamic (CT) cell to a layer 6 corticocortical (CC) cell, exhibited facilitation (average 2nd EPSPs colour coded for average amplitude). B: Average EPSPs peak amplitude was plotted against the preceding interspike interval, and fitted with monoexponential functions (dotted curves) to evaluate the apparent speed of recovery from facilitation ($\tau$, time constant; $r^2$, correlation coefficient). The thick dashed black line indicates the average amplitude of the EPSP triggered by the first action potential (1st EPSP).
displayed a time constant of 76 ms, while the recovery process for the 3\textsuperscript{rd} EPSP appeared faster with a time constant of 42 ms.

### 3.2.1.3 Post-tetanic potentiation

In figure 3.6, a connection between pyramidal cells in layer 4 is illustrated. The presynaptic and postsynaptic responses to current injection and their firing patterns are shown on the left hand side of figure 3.6 A. Both cells showed different degrees of burst adapting characteristics typical of layer 4, with the presynaptic cell showing a tighter initial burst of action potentials, together with a more weakly adapting pattern. The right hand side of figure 3.6 A shows the average EPSP triggered by single presynaptic action potentials. The peak amplitude for this average was 0.80 mV, its rise time from 10 to 90 % of peak amplitude was 0.90 ms and width at half amplitude was 8.0 ms.

The peak amplitude of the average single spike EPSP was dependant on the ongoing level of presynaptic activity (figure 3.6 B). Single spike EPSPs that followed trains of spikes (at an interval of 3 seconds) were, on average, larger than EPSPs that followed single spikes. This connection therefore displayed post-tetanic potentiation.
Figure 3.6: Electrophysiological characteristics of an excitatory connection onto an adapting cell in layer 4 of the neocortex. A: The responses to current injections (bottom) of the presynaptic (top) and postsynaptic (middle) cells are shown (left hand side). The average EPSP (green) elicited in response to a single presynaptic action potential (black) is shown (right hand side): amplitude, 0.80 mV; width at half amplitude, 8.00 ms; rise time, 0.90 ms. B: This connection displayed post-tetanic potentiation, single EPSPs that followed brief trains of action potentials were larger than those that followed single spikes. Error bars report the standard deviation. Student t-test, *: P<0.1; **: P<0.05.
3.2.2 Complex components in synaptic short term dynamics

The range of interspike intervals elicited presynaptically was used to map the characteristics of the postsynaptic responses in more detail.

3.2.2.1 Two-dimensional plots for the amplitude of later EPSPs

In figure 3.7, the single sweep measures of the amplitude of the second EPSP from the connection presented in figure 3.6 are plotted as a function of both the amplitude of the preceding (1st) EPSP and the preceding interspike interval, smoothed and represented by colour scale. The coordinates of the individual measurements are superimposed (small white dots). The amplitude of neighbouring EPSPs in the parameter space defined by the preceding EPSP amplitude and the preceding interspike interval are locally averaged (two-dimensional interpolation) to reveal trends in the data.

In the example of figure 3.7, as in all pairs for which sufficient data could be collected, the two-dimensional interpolation of later EPSPs displayed a stronger dependency upon the preceding interspike interval than upon the amplitude of the preceding EPSP. This is shown by the emergence of bands of amplitude (i.e. colour) similarly oriented perpendicularly to the interspike interval axis and parallel to the preceding EPSP amplitude axis.

The same representation can be used to study the dependency of the amplitude of EPSPs elicited by later action potentials in the train on other combinations of variables.

Thus the amplitude of the 3rd and 4th EPSPs for the layer 4 connection introduced in figures 3.6 and 3.7 are plotted in figure 3.8 (A-D and E-F respectively). The stronger dependency of EPSP amplitude upon interspike interval than upon preceding EPSP amplitude is again apparent in the subplots A, B and F, as, in all instances, bands of amplitude similarity
Figure 3.7: Relative dependency of EPSP amplitude on the preceding EPSP amplitude and the preceding interspike interval. Single sweep measurements of the peak amplitude of the 2nd EPSP were collected for the layer 4 connection introduced in figure 3.7. The interpolation of these measures against both the preceding EPSP amplitude and preceding interspike interval is shown here colour coded. The coordinates of the individual measures are indicated by the overlapping white dots. The local density of these points was used to manually define a shading area (dark) over the regions where the interpolation would reflect trends present in the data as opposed to algorithmic artefacts. Such plots usually indicate a stronger dependency of the EPSP amplitude over the preceding interspike interval than over the amplitude of the preceding EPSP, in displaying bands of amplitude similarity oriented parallel to the axis for that latter variable.
Figure 3.8: Relative dependency of EPSP amplitude on the preceding EPSP amplitudes and/or the preceding interspike intervals. Single sweep measurements of the peak amplitude of the 3rd (A-D) and 4th (E, F) EPSP were collected for the layer 4 connection introduced in figure 3.7 and 3.8. The interpolation of these measures against both the preceding EPSP amplitudes and/or preceding interspike intervals are shown here colour coded (A-F). Such plots usually indicate a stronger dependency of the EPSP amplitude over the preceding interspike interval than over the amplitude of the preceding EPSP, in displaying bands of amplitude similarity oriented parallel to the axis for that latter variable. These allow the visual identification of relevant data subsets based on the density of points at the intersection of the different variables.
emerge, oriented parallel to the amplitude axis and perpendicular to the interspike interval axis.

The remaining three graphs in figure 3.8 display similarly constructed two-dimensional interpolations of EPSP amplitude, but plotted against combinations of variables of the same kind, either the amplitudes of preceding EPSPs, or preceding interspike intervals. In such parameter space, the interpolations do not display bands of amplitude similarity. Rather, the regions of amplitude similarity appear to define more pocket-like areas, indicating that certain combinations of interspike intervals predispose the connection to generate larger or smaller events (figure 3.8 C-E).

### 3.2.2.2 Time course of successive EPSPs amplitude

Results obtained from the recording of a connection between pyramidal cells in layer 2 are presented in figure 3.9. The reconstruction of the two cell bodies and dendritic trees were completed (figure 3.9 A). These cells had short apical dendrite main trunks that bifurcated in sparse dendritic tufts of elongated branches in the transitional region between layers 1 and 2. The presynaptic (black) cell had distinctively fewer and shorter basal dendrites than its postsynaptic partner (red).

Average traces of the EPSP elicited by the second action potentials in the presynaptic trains, at different preceding interspike intervals, illustrate the short term dynamics displayed by the peak amplitude (colour coded) for this connection (figure 3.9 B).

The short term dynamics for EPSPs elicited by later action potentials in the presynaptic trains can be seen in plots of the smoothed (moving average over more than 20 points) single sweep measures of EPSP amplitude against preceding interspike interval (figure 3.9 C).
Figure 3.9: Morphological and electrophysiological characteristics of an excitatory connection between two pyramidal cells in layer 2 of the neocortex. A: Reconstruction of the cell bodies and dendritic trees of the recorded cells. B: Average traces of the second EPSPs at different interspike intervals, colour coded according to their peak amplitude. C: Smoothed (local average over more than 20 points) single sweep measures of EPSP amplitude plotted against preceding interspike interval allows the visualisation of the changes in EPSP amplitude with increasing interspike intervals. Dashed horizontal line indicates the mean amplitude of the first EPSP.
definition of relevant subsets could be guided by screening the time course of
the moving first derivative of the amplitudes using a window size similar to
the one used for smoothed amplitude, and selecting for its flatter portions
(moving first derivative closer to zero), with the further constraint of a minimal
subset size usually fixed at 30 points.

Additionally, subsets of first and single EPSP amplitudes were defined
on considerations of different levels of post-tetanic potentiation (see
section 3.2.1.3).

3.2.2.3 CV² indication on the locus of synaptic changes

The CV² method relies on the simple binomial model of synaptic
release. In a connection conceived as consisting of a number of release sites
(n) each having an average probability (p) of independently releasing
transmitter, and a quantal amplitude (q). The mean amplitude (M) of the
resultant composite postsynaptic responses is equal to the product n.p.q,
while the coefficient of variation of the amplitude (CV) is independent of q
(see section 2.4.1.3).

This is classically visualised by plotting CV², the inverse ratio of the
squared CV of the response in one release condition normalised to the
squared CV of the response in a reference condition, as a function of the
mean amplitude of the response in the release condition normalised to the
mean amplitude in the reference condition (see section 2.4.1.3).

The independence of CV upon the quantal size results in that, changes
that affect only q provide data points sharing the same ordinate value on the
graph, i.e. aligned horizontally (at ordinate 1 as the values are normalised).
Changes affecting only the probability of release (assumed to be of
presynaptic origin) provide data points that are situated below (decreased
Figure 3.10: Subsets of EPSP amplitudes with statistics indicative of presynaptic changes in release conditions. A: Smoothed single sweep measures of EPSP amplitude are plotted against interspike interval for the layer 4 connection introduced in figure 3.6. B: Normalised CV² values against normalised mean amplitude from subsets of single sweep measures selected in the peaks and troughs of the smoothed time course of amplitude for successive EPSPs (and at different levels of post-tetanic potentiation for the first EPSP) fall into the region indicative of a predominantly presynaptic origin for these differences in mean EPSP amplitude (region I).
mean) or above (increased mean) the identity line (region I in figure 3.10 B), because the resulting change in CV$^2$ is greater than the change in M. Changes that affect only the number of release sites ($n$) provide data points situated on the identity line. The complementary region (region II in figure 3.10 B) corresponds to the mixed case of joint contribution from pre- and post- synaptic components.

Therefore, under the assumptions of the model (see section 2.4.1.3), the prevalent or relative contribution of these parameters to the changes in synaptic efficacy, and thus the putative pre- or post- synaptic locus of these, can be assessed depending on the relative location of these data points on the graph.

The data from the defined subsets of amplitudes for the layer 4 connection were plotted according to the CV$^2$ method in figure 3.10 B. All data points fell within the region I of the parameter space, relative to the CV and amplitude of the reference condition (subset of 1st EPSPs without post-tetanic potentiation, black dot at coordinates (1,1) in figure 3.10 B).

This result was an indication that the changes in mean amplitude between the selected subsets of EPSPs had a presynaptic origin that could be presumed to involve changes in the probability of release.

Moreover these changes were similarly attributed to $p$ for subsets from three different dynamic conditions (figure 3.10 B): post-tetanic potentiation of the single and first EPSPs (black dots), depression of the second to fifth EPSPs (lower left quadrant) and “slight facilitation” of some of the second EPSPs (upper right quadrant); suggesting that the mechanisms underlying short term dynamics would operate through the regulation of release probability at some connections.
3.2.3.3 Estimates of binomial parameters

Four relationships between statistical measures of the variability in the postsynaptic response amplitude and the corresponding mean amplitude were used to obtain functional estimates of the binomial parameters (figure 3.11).

The Coefficient of Variation (CV), Failure rate (F), Variance (V) and the ratio of the Variance to the Mean amplitude (V/M) of the selected subsets of EPSP amplitudes (see preceding sections in 3.3.1) were plotted against their Mean amplitude (M), and nonlinear least square fitting methods used to identify the particular sets of the parameters $n$, number of release sites, and $q$, quantal size, that yielded the closest fit to the data for each relationship (figure 3.11 A-D).

The four relationships were derived from the premise that the differences between the statistical coordinates of the defined subsets arose primarily from different probabilities of release. Importantly a range of such probability conditions needs to be represented for the fitting methods to be applicable. In this example, both these conditions were fulfilled (see section 3.3.1.2).

In the example of the layer 4 excitatory connection presented in figure 3.11, three of the fitting methods provided very close estimates of the binomial parameters that described the range of synaptic conditions displayed according to the simple binomial model: CV against M, Variance against M and Variance/M against M methods provided functional estimates of 2.3 as the number of release sites and a quantal size of 0.45 mV (figure 3.11 A, C and D). The Failure rate against M method (figure 3.11 B) provided a lower estimate for the number of release sites (1.67), together with a larger value for the quantal size of 0.59 mV.
Figure 3.11: Fitting the statistics of EPSPs amplitude subsets with binomial relationships provides functional estimates for a synaptic connection. Four statistics of the amplitude subsets of successive EPSPs (see key) from the layer 4 connection examined in figure 3.11, are plotted against their Mean amplitude: Coefficient of Variation (A), Failure rate (B), Variance (C) and the ratio of the Variance to the Mean amplitude (D). Dashed black curves illustrate the outcome of applying nonlinear least square fitting for the corresponding relationships predicted by the simple binomial model, with $n$ and $q$ as adjusting parameters. The respective estimates for these parameters are also indicated in each case, together with the consecutive estimate of the initial probability of release ($p$) and the correlation coefficient of the fit ($r^2$).
All four methods resulted in high correlation coefficients \( r^2 \) ("R square") indicating how much of the variation within the data was captured. The Variance against M method being the lowest with a R-square of 0.76, while the CV against M and Failure rate against M methods yielded values above 0.9, and the Variance/M against M method resulted in a R-square of 0.85.

The mean initial probability of release corresponding to these estimates could be calculated from the average mean amplitude of the first EPSP (see section 2.4.1.4). All four methods indicated a high initial probability, ranging from 0.73 to 0.79.

These estimates therefore indicated that the synaptic short term dynamics displayed by this connection could be described by a small number of release sites with a high initial probability of release generating quantal responses in the order of 0.5 mV.
3.3 BEHAVIOUR OF DYNAMIC STOCHASTIC SYNAPSES

To better understand the consequences and behaviours of a strictly
to better understand the consequences and behaviours of a strictly
binomial synaptic model in the context of our experimental protocols and data-
binomial synaptic model in the context of our experimental protocols and data-
analysis, a dynamic-stochastic model of synaptic transmission was developed.
analysis, a dynamic-stochastic model of synaptic transmission was developed.
The way in which interspike interval was suggested to influence the probability
The way in which interspike interval was suggested to influence the probability
of release was simulated for deterministic and stochastic models and the
of release was simulated for deterministic and stochastic models and the
outcomes compared.

3.3.1 Dynamic and stochastic behaviours

The available specifications of release site dynamics in the dynamic-
The available specifications of release site dynamics in the dynamic-
stochastic model (see section 2.6.6) are shown as a sequential diagram in figure
stochastic model (see section 2.6.6) are shown as a sequential diagram in figure
3.12, A. The synaptic connection to be simulated was defined by a integer
3.12, A. The synaptic connection to be simulated was defined by a integer
number of individual release sites. For the data discussed in this section, each
number of individual release sites. For the data discussed in this section, each
site could be specified as releasing in a stochastic or deterministic fashion.
site could be specified as releasing in a stochastic or deterministic fashion.
Further, the dynamics affecting the probability of release ($p$) at each site could
Further, the dynamics affecting the probability of release ($p$) at each site could
be specified independently as release-dependent (transition in the current value
be specified independently as release-dependent (transition in the current value
of the variable when release happens), or release-independent (transition
of the variable when release happens), or release-independent (transition
happens irrespective of the stochastic result). In most simulations depression
happens irrespective of the stochastic result). In most simulations depression
was simulated by setting $p$ to half of its immediately preceding value. As in
was simulated by setting $p$ to half of its immediately preceding value. As in
Tsodyks et al. (1998), recovery from depression followed a double exponential
tSodyks et al. (1998), recovery from depression followed a double exponential
time course.
time course.

When the parameters and stimulation conditions used in Tsodyks et al.
When the parameters and stimulation conditions used in Tsodyks et al.
(1998) were used in simulations of the dynamic-stochastic model (figure 3.12 B,
(1998) were used in simulations of the dynamic-stochastic model (figure 3.12 B,
blue traces), the dynamic behaviour exemplified by the average postsynaptic
blue traces), the dynamic behaviour exemplified by the average postsynaptic
voltage trace (black) could be replicated (red).
Figure 3.12: Dynamics affecting the probability of release at individual release sites in the dynamic-stochastic model. A: Available specifications of release site dynamics are shown as a sequential diagram. B: The dynamics displayed by the deterministic model (in black) of excitatory cortical connections (Tsodyks et al., 1998) could be replicated in average traces (in red), but not single sweeps (in blue) from simulations of the dynamic-stochastic model. C: The statistics relative to EPSP amplitude from simulations of the dynamic-stochastic model (running average in green, running standard deviation as grey error bars) matched binomial predictions (dotted).
The statistics relative to EPSP amplitude from subsets generated at precise interspike intervals in simulations of the dynamic-stochastic model matched theoretical predictions (figure 3.12 C). Over the course of recovery from depression, the average EPSP amplitude (green) and standard deviation (error bars) followed a trajectory similar to the equivalent deterministic model (dotted black curve), with a level of variability consistent with the binomial model (dotted orange curves) based on the particular values of initial release probability, depression ratio and recovery time constants used.

A simulation of the deterministic model was performed. The distributions of all preceding interspike intervals were uniform over the time window of interest, between 5 and 120 ms. The effect of the immediately preceding intervals on the EPSP amplitude irrespective of the duration of earlier intervals is illustrated in figure 3.13, A. The cumulative effect of incomplete recovery from depression during preceding intervals was largely removed by the inclusion of long 1st and 2nd interspike intervals. The cumulative effect can also be seen to decline with larger preceding interspike intervals, as the three curves converge at the longest interspike intervals. The small cumulative effect of depression is the result of short time constants for recovery in this example.

The dependency of EPSP amplitude upon preceding activity in simulations of the deterministic model is plotted colour coded in figure 3.13, B1-B4. The relative influence of the determinants of EPSP amplitude is indicated by the orientations of amplitude similarity bands. Stronger dependency upon the preceding interspike interval than upon the preceding EPSP amplitude is apparent (figure 3.13 B1). In addition, a stronger dependency on the immediately preceding interspike interval than on earlier intervals was apparent (figure 3.13 B2 and B4). In this example, the amplitudes of preceding EPSPs (figure 3.13 B3) were not strong determinants of the following EPSP amplitude. Since this is a deterministic model, the apparent correlation between 2nd and 3rd
Figure 3.13: Short term dynamics in the deterministic model. The dynamic behaviour observed when release is not considered stochastic is represented. **A**: Smoothed time courses of recovery from depression for successive EPSPs (see colour key) in simulations using deterministic release. **B**: Synaptic dynamics in simulations with deterministic release are shown as plots, colour coded for the amplitude of the 3rd and 4th EPSPs in the train. Shaded areas correspond to regions of the plots where the scarcity of measurements (white dots) could not allow meaningful interpolation.
EPSP amplitudes is the result of the influence of the interspike interval on both parameters.

### 3.3.2 Simulations of dynamic-stochastic synaptic connections

Two specifications of the dynamic-stochastic model were designed to implement connections with parameter sets at opposite ends of the spectrum derived from experimental data (table 3.2).

The first connection type was specified as using a small number of release sites (4) with a large quantal amplitude (0.71 mV). The second connection type was specified as using a larger number of release sites (20) with a smaller quantal amplitude (0.19 mV). Initial probability of release was 0.6 in both types, insuring that the average 1st EPSP amplitudes would be of similar magnitudes.

Both connection types were simulated following either a fast ($\tau_{\text{rec}} = 10$ ms, $\tau_{\text{in}} = 3$ ms) or slower ($\tau_{\text{rec}} = 50$ ms, $\tau_{\text{in}} = 3$ ms) recovery from depression (see figure 2.6 and section 2.6.6).

Smoothed time courses of recovery obtained from examples of the dynamic-stochastic model including stochastic release are shown in figures 3.14-3.16. Pronounced peaks and troughs in the running EPSP amplitude were present over the course of recovery irrespective of the specifications considered. The 3rd EPSP time courses (figure 3.15) displayed an artefactual interrupted distribution of interspike intervals, that was not seen to induce systematic differences in later analysis. No clear differences could readily be seen between sample outcomes from simulations of the release-independent (A) and release-dependent (B and C) specifications in these examples.

Figure 3.17 shows the dependency of the EPSP amplitude upon preceding activity in simulations of both connection types (few release sites in A and numerous sites in B). The assumption of a strictly quantal release explains the
Figure 3.14: Smoothed single sweep measures of bootstrap samples of 2\textsuperscript{nd} EPSPs amplitude from simulations of the dynamic-stochastic model. Smoothed single sweep measures of amplitude are plotted against the preceding interspike interval for the 2\textsuperscript{nd} EPSPs. Release-independent (A) and release-dependent (B and C) dynamics were simulated with time constants 50 ms (A1 and B) or 10 ms (A2 and C). Two configurations were considered: 4 release sites using a quantal amplitude of 0.71 mV (A1-C1) or 20 release sites using a quantal amplitude of 0.19 mV (A2-C2). Initial release probability was 0.6 for both types. Smoothed time courses showed interruptions in the recovery from depression.
Figure 3.15: Smoothed single sweep measures of bootstrap samples of 3rd EPSPs amplitude from simulations of the dynamic-stochastic model. Smoothed single sweep measures of amplitude are plotted against the preceding interspike interval for the 3rd EPSPs. Release-independent (A) and release-dependent (B and C) dynamics were simulated with time constants 50 ms (A1 and B) or 10 ms (A2 and C). Two configurations were considered: 4 release sites using a quantal amplitude of 0.71 mV (A1-C1) or 20 release sites using a quantal amplitude of 0.19 mV (A2-C2). Initial release probability was 0.6 for both types. Note: interrupted interspike interval distributions are artefactual.
Figure 3.16: Smoothed single sweep measures of bootstrap samples of 4th EPSPs amplitude from simulations of the dynamic-stochastic model. Smoothed single sweep measures of amplitude are plotted against the preceding interspike interval for the 4th EPSPs. Release-independent (A) and release-dependent (B and C) dynamics were simulated with time constants 50 ms (A1 and B) or 10 ms (A2 and C). Two configurations were considered: 4 release sites using a quantal amplitude of 0.71 mV (A1-C1) or 20 release sites using a quantal amplitude of 0.19 mV (A2-C2). Initial release probability was 0.6 for both types. Smoothed time courses showed interruptions in the recovery from depression.
discrete distribution of EPSP amplitudes (white dots) in plots A1, B1, A3 and B3. Stronger dependency of EPSP amplitude upon preceding interspike interval than upon the preceding EPSP amplitude is apparent in this model. When the interplay of preceding interspike intervals was considered however (figure 3.17 A2 and B2), the EPSP amplitude did not display the stronger dependency upon immediately preceding interval that was seen in simulations with deterministic specifications (figure 3.13 B2).

3.3.3 Relative deviation from exponential recovery

The relative size of peaks and troughs in the smoothed time courses of recovery was evaluated for comparison of the two connection types.

300 bootstrap samples of 1000 sweeps were performed within the 3600 sweeps simulated. The distances between the smoothed amplitudes and the predicted exponential recovery were calculated for each sample, and the relative deviation measure was defined as the ratio of these distances to the corresponding point along the predicted exponential recovery. The cumulative distributions of relative deviation over all 300 samples for the 2nd, 3rd and 4th EPSP are shown in figure 3.18.

The extent of relative deviation detected by this procedure in simulations of the different models are shown in figure 3.18. Relative deviation in 2nd (green), 3rd (red) and 4th (blue) EPSP amplitudes were very similar within simulations of a given specification. Some deviation from the predicted exponential recovery could be detected in the case of the deterministic model (figure 3.18 A), providing an evaluation of the imprecision in the automated measurement procedure of simulated EPSPs (see section 2.6.6 and figure 3.13 A). Larger values of relative deviation were detected from simulations with stochastic release specifications (figure 3.18 B and C; \( P<0.01 \), Kolmogorov-Smirnov test),
Figure 3.17: Sample dynamics in simulations of two configurations of the stochastic-dynamic model. Data obtained from a single random sample of 1000 sweeps in simulations of the connection types with few (A) or numerous (B) release sites with release dependent dynamics are shown. The 2nd (A1, B1) and 3rd (A2-3, B2-3) EPSP amplitudes are plotted against combinations of preceding interspike intervals and/or preceding EPSP amplitude. Note the discrete distribution of EPSP amplitudes in A1, B1, A3 and B3, due to the assumption of a strictly quantal release in the model. Stronger dependency upon one or the other parameter is indicated by the emergence of amplitude similarity bands.
Figure 3.18: Distributions of running average amplitudes relative deviation from exponential recovery in simulations of different dynamic models. Simulations of 3600 sweeps at a range of interspike intervals were performed for: the deterministic model (A), the numerous release sites connection type (B) and the few release sites connection type (C). The relative distance of successive EPSPs (see colour key) running average amplitude from predicted exponential recovery were collected over 300 bootstrap resampling of 1000 sweeps each. The distributions of the corresponding values of deviation relative to a smoothed exponential recovery are plotted.
indicating that the size of peaks and troughs in the simulations was influenced by stochasticity.

Further, larger values of relative deviation were provided in simulations of the stochastic model for the connection type with few release sites (figure 3.18 C) than for the connection type with numerous release sites (figure 3.18 B; \( P<0.01 \), Kolmogorov-Smirnov test). This confirmed that larger peaks and troughs over the time course of recovery could be indicative of a connection with a smaller number of release sites and a larger mean quantal amplitude.

Neither the release-dependency status of \( p \) dynamics nor the values of the recovery time constant used influenced these deviations from the smooth exponential decay seen in the deterministic model (\( P>0.1 \), Kolmogorov-Smirnov test). This indicated that these two parameters did not influence the size of peaks and troughs in the smoothed amplitude time courses.

### 3.3.4 Moving distributions of EPSP amplitude

The plots shown in figures 3.19 and 3.20, indicate that peaks and troughs in the sampled smoothed amplitudes are influenced by random and transitory shifts in the corresponding moving distributions of amplitudes. The sharpness in these transitions can be expected to result in part from the strictly quantal nature of the model simulated. The sampled moving distributions of EPSP amplitude (plots A1, B1 and C1) were compared with the idealized distributions obtained from the predicted values of the probability of release during its exponential recovery from depression (plotted in A2, B2 and C2) by Kolmogorov-Smirnov test. The black points indicate those at which the two distributions did not differ significantly (\( P>0.1 \), KS-test). With a larger \( n \) and smaller \( q \) the deviation from a smooth, exponential decay is reduced.
Figure 3.19: Moving distributions of EPSP amplitude in simulations of the dynamic-stochastic model for the few release sites connection type. Data from a single random sample of 1000 sweeps of simulations are shown. The moving distributions of 2\textsuperscript{nd} (A), 3\textsuperscript{rd} (B) and 4\textsuperscript{th} (C) EPSP amplitudes are colour coded in the sampled (A1-C1) and predicted (A2-C2) situations. Smoothed EPSP amplitudes from the sample are shown as superimposed dots. Black dots indicate the points at which the moving distribution from the sample was not significantly different from the predicted one through Kolmogorov-Smirnov test (P>0.1). Note: interrupted interspike interval distribution in B is artefactual.
Figure 3.20: Moving distributions of EPSP amplitude in simulations of the dynamic-stochastic model for the numerous release sites connection type. Data from a single random sample of 1000 sweeps of simulations are shown. The moving distributions of 2nd (A), 3rd (B) and 4th (C) EPSP amplitudes are colour coded in the sampled (A1-C1) and predicted (A2-C2) situations. Smoothed amplitudes from the sample are shown as superimposed dots. Black dots indicate the points at which the moving distribution from the sample was not significatively different from the predicted one through Kolmogorov-Smirnov test (P<0.01). Note: interrupted interspike interval distribution in B is artefactual.
3.3.5 Stationary subsets provide optimal binomial statistics

Subsets of experimental data were typically selected in the peaks and troughs of smoothed recovery. To investigate whether this assumptions was appropriate in the case of simulations of the dynamic-stochastic model, bootstrap resampling was used. The normalised distance to target binomial statistics (average amplitude and variance), was calculated based on the predicted recovery in probability of release. The average values of normalised distance over 300 samples of 1000 sweeps each are plotted (colour coded) against their moving first derivative for a range of subset sizes between 2 and 40 consecutive points. The results regarding the few release sites and numerous release sites connection types are shown respectively in figures 3.21 and 3.22 for the 2nd (A), 3rd (B) and 4th (C) EPSPs.

The normalised distance from target binomial statistics was found to converge with increasing subset size but only close to null values of the moving first derivative. In these simulations, larger subsets were more likely to provide statistics relevant for fluctuation analysis when they were selected from stationary portions of the smoothed amplitude time courses. Furthermore, the null moving first derivative was usually characterised by the lowest normalised distance for subset sizes larger than 10. This was particularly discernible in the connection type with few release sites, although better estimated values (smaller normalised distance) were reached in the connection type with numerous release sites. Altogether this confirmed that the selection of stationary portions of the smoothed amplitude time courses was an appropriate approach regarding the selection of subsets for further analysis.

In both specifications, the convergence towards smaller relative distance around null moving first derivative values with increased subset size appeared asymmetrical. Optimal statistical estimates resulted from a larger range of
Figure 3.21: Null moving derivative of smoothed EPSP amplitude time course indicates optimal subsets statistics. Simulations of the dynamic-stochastic model with few release sites connection specifications were performed. Measures of the first derivative and the relative distance (see excerpt) to the predicted average amplitude and variance of running subsets of 2nd (A), 3rd (B) and 4th (C) EPSPs were collected for a range of subset sizes. Average values from 300 bootstrap resampling of 1000 sweeps each are shown colour coded.
Figure 3.22: Null moving derivative of smoothed EPSP amplitude time course indicates optimal subsets statistics. Simulations of the dynamic-stochastic model with numerous release sites connection specifications were performed. Measures of the first derivative and the relative distance (see excerpt) to the predicted average amplitude and variance of running subsets of 2\textsuperscript{nd} (A), 3\textsuperscript{rd} (B) and 4\textsuperscript{th} (C) EPSPs were collected for a range of subset sizes. Average values from 300 bootstrap resampling of 1000 sweeps each are shown colour coded.
negative first derivative values than positive ones. This was consistent with a
tendency visualised in figure 3.19 for decreasing portions of the smoothed
amplitudes time courses to correspond to distributions of amplitudes more
similar to the idealised binomial distribution (black dots).

3.3.6 Frequency profiles

To investigate whether peaks and troughs in smoothed amplitude time
courses were organised as oscillations with any degree of regularity, bootstrap
resampling considering the power spectrum of the relative deviation from
exponential recovery was performed, and its average profile was plotted
(figure 3.23) for 2\textsuperscript{nd} to 5\textsuperscript{th} EPSP (see colour key). This allowed the power
distribution of the relative deviation across all samples at a particular frequency
to be compared (colour coded stars; \(P<0.01\), Kolmogorov-Smirnov test) with its
distribution over the neighbouring 5 Hz band (\textit{i.e.} power distributions over
frequencies -2, -1, +1 and +2 Hz away from the one considered). The ability of
this procedure to detect a sinusoidal signal was tested by embedding a sinusoid
in the smoothed time course of recovery obtained from the 2\textsuperscript{nd} EPSPs in
simulations of the stochastic-dynamic model with deterministic specifications
(dotted grey curve). The resulting frequency profile did show a large peak at the
specified frequency which was detected in the power distribution across
bootstrap samples as being significatively different from its neighbouring 5 Hz
band (\(P<0.01\), Kolmogorov-Smirnov test).

Simulations of the deterministic model allowed the frequency profiles
obtained when release was not stochastic to be established (figure 3.23 A).
Although bootstrap power distributions at particular frequencies that differed
from their neighbouring 5 Hz band could be detected, no clear pattern of
oscillation generation could be identified.
Figure 3.23: Average power spectra from bootstrap sampling of smoothed time courses of successive EPSPs amplitude recovery in different configurations of the dynamic-stochastic model. Power spectra of the 2\textsuperscript{nd} to 5\textsuperscript{th} EPSP (see colour key) smoothed time course of recovery are plotted. Synaptic models simulated were deterministic (A), stochastic with release independent dynamics (B), and stochastic with release dependent dynamics following different time constants of recovery (C, 50 ms; D, 10 ms). Two configurations were considered: 4 release sites and a quantal amplitude of 0.71 mV (A and B1-D1) or 20 release sites using a quantal amplitude of 0.19 mV (B2-D2). Colour coded stars indicate differences in distribution of power between a particular frequency and its neighbouring 5Hz band through Kolmogorov-Smirnov test at P<0.01.
When stochastic release specifications were considered (plots B-D), the frequency profiles of the smoothed amplitudes of 2\textsuperscript{nd} to 5\textsuperscript{th} EPSPs displayed increased power over the 1-100 Hz range compared with the deterministic case. The connection type with fewer release sites provided larger power values than the connection type with many release sites, over this same range of frequencies (plots B1-D1 compared with B2-D2). Again, although bootstrap power distributions at particular frequencies could be detected that differed from their neighbouring 5 Hz band, no clear pattern of oscillation generation over the successive EPSPs could be identified irrespective of the specifications considered. Another common feature obtained from these simulations appeared to be a sharper drop in power at frequencies around 100 Hz specific to the 2\textsuperscript{nd} EPSP time courses (green curves). No clear difference in frequency profiles could be identified in comparing release-independent (B) and release-dependent (C and D) specifications, nor in comparing specifications with longer (C) or shorter (D) recovery time constants.

This indicated that a stochastic-dynamic mode of release alone was not sufficient to induce the peaks and troughs in the smoothed time course of EPSPs amplitude to be organised into specific frequencies of oscillations. The seemingly indiscriminate increase in power over a range of frequencies between 1 and 100 Hz in smoothed amplitude time courses of 2\textsuperscript{nd} to 5\textsuperscript{th} EPSPs from simulations however, indicated that the mere appearance of peaks and troughs resulted from the stochastic nature of release.
3.4 MONTE CARLO SIMULATIONS OF BINOMIAL MODELS

The same statistical relationships used for the assessment of functional parameters at experimentally recorded cortical connections were studied in Monte-Carlo simulations of the simple binomial model and models in which the release sites were not identical. This made it possible to assess to what extent methods based on the simple binomial model could apply to more complex schemes of synaptic transmission.

3.4.1 Convergence of binomial statistics

The evaluation of the functional parameters was performed by fitting the relationships between two statistics (mean and variability) from samples of amplitudes generated by binomial models of synaptic transmission. The reliability of the statistics can be expected to be dependent on the size of the samples considered. It was therefore valuable to gain insight into the expected convergence rate of these statistics before performing the computationally expensive Monte-Carlo simulations associated with fitting methods.

This approach is reported in figure 3.24 for the mean (A1 and A2) and the Coefficient of Variation (CV; B1 and B2). In figure 3.24 A1 and B1, a Monte-Carlo simulation at 500 repetitions of 300 draws each from one particular simple binomial model instance (n=4 and p=0.5) was conducted and the cumulative statistics of each repetition along the number of draws (sample size) monitored. The larger (in red) and lower (in blue) values of the cumulative statistics exhibited across all 500 repetitions were plotted against the increasing sample size (number of binomial draws).

Convergence was defined as the smallest sample size at which 90% (second vertical dotted grey line) of the repetitions would present the given cumulative statistic as falling within a convergence range from the theoretical
Figure 3.24: Sample size required for convergence of statistics in simulations of a simple binomial model. The highest (red) and lowest (blue) values of cumulative mean (A1) or cumulative coefficient of variation (CV, B1) from 500 repetitions in a Monte Carlo simulation of the simple binomial model with $n=4$ and $p=0.5$ are plotted against the increasing size of the sample. Horizontal dashed lines indicate the convergence range, defined as ±5% of the maximum range around the theoretical expected value for the statistic. Vertical dotted lines indicate the smallest sample size at which 50 then 90% of the 500 repetitions presented a cumulative statistic falling within the convergence range. The sample size required for each statistic (A2: Mean; B2: CV) in the 90% case has been mapped against the binomial parameters $n$ (x axis) and $p$ (y axis). This is reported by boundary lines of sample size (see labels) required for the convergence of the respective statistic.
expected value. This convergence range was defined as ±5% of the maximal range for the statistic: the lower limit being zero for both M and CV (corresponding to two initial failures, and two identical initial release outcomes respectively), the upper limit comes from extreme pairs of draws \( k=n \) twice i.e. release happens at all sites on both the first two draws in the case of M, and the pair \( k=0 \) and \( k=n \) i.e. exactly one failure and one “full release” in the case of CV). Also indicated in A1 and B1 is the 50% limit (leftmost vertical dotted grey line), indicating the sample size beyond which more than half the repetitions exhibited a cumulative statistic within the convergence range from the expected value.

Applying the procedures presented in A1 and B1, the sample size required for 90% convergence of each statistic (figure 3.24, A2 and B2 for mean and CV respectively) was mapped as a function of the binomial parameters \( n \) (x-axis) and \( p \) (y-axis). This is reported as contour plots with boundary lines of the specific sample sizes required for the 90% convergence of the respective cumulative statistic.

By considering the results obtained for models with either \( n=4 \) or \( n=8 \) (representative of experimental connection classes), it was possible to confirm that in order to ensure convergence of CV across the range of probabilities considered (restricted for biological relevance), a sample size of 300 draws was suitable (figure 3.24 D). This ensured that any deviation in the outcomes of the fitting procedures when applied to similar Monte-Carlo simulations would be due to the differences in binomial models, rather than scatter in simulated statistics. Simulations for experimentally more realistic sample sizes of 60 draws were also conducted, to establish the impact on parameter estimation of partial convergence of the statistics in such circumstances.
3.4.2 Behaviour of complex binomial models

3.4.2.1 Distribution of statistics from a complex binomial model

Monte-Carlo simulations of 300 draws were used to compare the outcomes of simple and complex binomial models. This approach is presented in figure 3.25, focussing on the complex binomial model in which individual p and q values were correlated across sites (see section 2.6.3) because it was found in subsequent analysis to be the one that deviated most strongly from the simple model case. Nevertheless, when the statistics from 100 simulations of this complex model (in black) were superimposed with their counterpart from the simple model with identical mean parameters (in grey), a large proportion of the outcomes appeared overlaid.

This was particularly true for the “Failure rate against M” relation (B). Some deviation was displayed by the other three statistical relationships (A, C and D).

3.4.2.2 Distributions from different functional parameters

Changes resulting from non-uniformity of the release sites characteristics (section 3.4.2.1) could be compared with the case of changing the mean value of a single binomial parameter (figure 3.26). The outcome of two such changes from the basal case (n=4, mean q=0.5, in black, same as figure 3.25) are depicted. One with binomial n increased from 4 to 5 (in red), the other one with mean q decreased from 0.5 to 0.4 (in green).

When the binomial parameters were changed, the shifts in the relationships between variability and M were larger. In comparison to figure 3.25, the extent of, and the trends in shifts from the basal case were more powerful, with the exception of increasing n in the “failures against M” relationship (figure 3.26 B).
Figure 3.25: Distributions of statistics from Monte-Carlo simulations of a simple and a complex binomial model. The four statistics of samples generated by Monte Carlo simulation of a simple (grey) and a complex (black) binomial model, with identical mean quantal amplitude and number of release sites ($n=4$, mean $q=0.5$), are plotted against their Mean amplitude: Coefficient of Variation (A), Failure rate (B), Variance (C) and the ratio of the Variance to the Mean amplitude (D). Each dot is the result of a 300 draws sample. At each of 10 $p$-conditions, the results from a 100 samples are shown. The general overlap of the distributions predicts that the corresponding fitting methods would provide similar functional estimates in the simple and complex cases.
Figure 3.26: Distributions of statistics from Monte Carlo simulations of a complex binomial model with different mean quantal amplitude or number of release sites. The four statistics of samples generated by Monte Carlo simulation of a complex binomial model are plotted against their mean amplitude: Coefficient of Variation (A), Failure rate (B), Variance (C) and the ratio of the Variance to the Mean amplitude (D). The outcomes of three different sets of parameters are compared, $n=4$ and mean $q=0.5$ (in black), $n=5$ and mean $q=0.5$ (in red), $n=4$ and mean $q=0.4$ (in green). Each dot is the result of a 300 draws sample. At each of 10 $p$-conditions, the results from a 100 samples are shown. Clear distinction in relationship profiles between the three cases predicts that, despite being implemented in a complex binomial model, binomial parameters can be distinguished by these fitting methods.
It can be expected that, only in cases of overlapping distributions of values, would the fitting methods provide similar functional estimates for different models. The regions of less overlap corresponded to high \( p \) conditions (figure 3.26), one exception being the distribution of statistics obtained from increasing \( n \) in the "failures against M" relationship (figure 3.26 B, compare red with black). In this situation, \( n \) (but not \( q \)) can be expected to be less consistently estimated.

Importantly however, the overall picture of deviations displayed in figure 3.14, still conforms to the equivalent changes considered in the simple binomial case (see section 2.4.1.4 and figure 2.4), despite the fact that these data were obtained from simulations of the complex binomial model that was found to disrupt most strongly the fitting methods (\( p \& q \) correlated).

### 3.4.2.3 Distributions from different complex binomial models

Next, consideration was given to identifying potentially specific statistical profiles for the different complex models simulated. Monte-Carlo simulations of each model were conducted, with 500 repetitions and a sample size of 300 draws, all with identical mean binomial parameters (see section 2.6.3 and table 2.1 as an example).

The means and standard deviations of the distribution of statistics (sample Mean, Coefficient of Variation, Variance, Variance/Mean, and Failure rate) were collected as illustrated in figure 3.27. This confirmed that the statistics obtained from simulations of all five complex models followed relationships similar to the one extracted from the simple binomial model as, over the range of release probability explored, the expected shapes for the relationships were conserved (see section 2.4.1.4 and figure 2.4). Namely, "CV against M" displayed a square root of an inverse function shape, "Failure rate against M" followed a geometric
Figure 3.27: Distributions of statistics from Monte Carlo simulations of different complex binomial models having the same average values of functional parameters. The mean (circles) and standard deviation (error bars) of the distributions in four statistics of samples generated by Monte Carlo simulation of complex binomials model are shown: Coefficient of Variation (A), Failure rate (B), Variance (C) and the ratio of the Variance to the Mean amplitude (D). The outcomes of five complex binomial models (see key) with identical mean parameters (n=4, mean q=0.5 and 10 p-conditions) are compared. Each point is the outcome of 500 samples of 300 draws. Little deviation in the relationship profiles were apparent between all complex models, predicting that fitting methods would provide similar functional estimates when applied to the outcomes of binomial models with identical mean parameters.
decay, “Variance against Mean” points laid along a parabola and “Variance/Mean against M” followed an inverse function. Moreover, the complex p&q correlated model in figure 3.27 (black) is the case represented in figure 3.25, in which the distributions of statistics from a smaller number of samples were compared with outcomes of the simple binomial model, and found to display similar and largely overlapping distribution patterns.

Furthermore the overlap between the distributions in figure 3.27 did not indicate any specific model for which a particular deviation from the classic simple relationships would be apparent.

As was expected from the results presented in figure 3.24, the amount of jitter in M across the range of p-values (n=4) was near constant, but the jitter in CV, F and V/M was larger for smaller values of p in all cases save for the Variance (vertical error bars in figure 3.27). In the latter case larger jitter was apparent at probability values closer to 0.5.

These results led to the expectation that applying the fitting methods to samples from these more complex models would not result in any dramatic divergence in the estimation of the functional parameters from their actual mean quantal amplitude and number of release sites.
3.4.3 Functional estimates for complex binomial models

To evaluate the amount of disruption in the performance of the fitting methods when challenged by simulations of more complex binomial models of synaptic release, Monte-Carlo simulation outcomes were systematically assessed by the four fitting methods (see sections 2.4.1.4 and 2.6.4).

3.4.3.1 Distributions of functional estimates

The results from this approach are shown in figure 3.28 as cumulative distributions of the parameter estimates ($n$ and $q$ in A and B respectively) provided by each fitting method (columns of graphs from left to right of figure 3.28) for all six binomial models (indicated by the colour of the curve, refer to key). For each parameter, two cases were distinguished, one for a range of low release probability conditions (first row: Aa-Ad and Ba-Bd), the other for a range of high $p$-conditions (second row: Ae-Ah and Be-Bh).

First, attention should be paid to the distributions in the simple binomial model case (curves in grey). The cumulative distribution profile in this case was typically sigmoid, reflecting the Gaussian shape of the underlying distribution, and reached half accumulation (median value) exactly at the original parameter values ($n=8$ and $q=1.00$). Thus, in most cases, parameter estimations from the fitting methods resulting from simple binomial theory were reliable when applied to the statistics generated by Monte Carlo simulations of the simple binomial model. These considerations validated the suitability of the random number generators used for Monte Carlo simulations.

Regarding the estimates of the number of release sites, $n$ (figure 3.28 A), the steepness of the cumulative distributions obtained from fitting simulation outcomes in the low and high $p$-conditions range were distinct (e.g. compare Aa and Ae). High $p$-conditions resulted in a steeper distribution i.e. a narrower
**Figure 3.28:** Distributions of functional estimates provided by four fitting methods applied to the outcomes of Monte Carlo simulations of six different binomial models with identical mean parameters. Cumulative distributions of the functional estimates of the number of release sites ($n$, $Aa$-$h$) and mean quantal size ($q$, $Ba$-$h$) provided by each fitting method (see the columns of graphs titles) when applied to the statistical outcomes of 500 repetitions from one simple and five complex binomial models (see colour key) over two probability of release ranges (high and low, see the rows of graphs titles). All models had 8 release sites and a mean quantal size of 1 [range across sites when applicable: 0.57-1.43]. Low $p$-conditions range from 0.16-0.24 (intersite) to 0.39-0.56, and high $p$-conditions range from 0.41-0.59 to 0.66-0.94. Three fitting methods provided similar estimation trends for all binomial models. The remaining method, Failure rate against Mean, was only accurate for $q$ estimation at low probability ranges.
range of estimates. This also occurred with the more complex models, and was
a sign of more consistency in parameter estimation.

The shape and steepness of the sigmoid curves obtained from the more
complex binomial models were usually comparable to the simple model case
within a range of $p$-conditions (e.g. figure 3.28 Aa-Ac). A correlation between the
parameters $p$ and $q$ across sites (either positive or negative, black and purple
respectively) appears to lead to steeper curves in high $p$-conditions
(e.g. figure 3.28 Ae). This observation allowed comparison of median values
presented by the distribution of estimates, as none decisively showed less
consistency in parameter estimation than the simple case.

The simulation of the complex model where only $p$ was varied across sites
(in green) provided statistics leading to the superposition of both estimated $n$
and estimated $q$ distributions with the simple binomial case (in grey). This
suggested that, within the limits fixed for the variability of $p$ in our simulations, no
disruption of parameter estimates resulted from this level of complexity in the
binomial model. A similar conclusion can be reached for the estimation of $n$
with simulations of the complex model where $p$ and $q$ values are anti-correlated (A, in
purple).

The situation for the remaining 3 complex models can be summarised as
follows: the complex model in which only $q$ varied across sites (in red) and the
model in which $p$ and $q$ varied but were uncorrelated across sites (in blue) gave
a distinguishable set of cumulative distributions, with median values of estimated
$n$ lower than the actual number of release sites (figure 3.28 Aa-Ac, Ae-Af) and
higher than the actual values for estimated $q$ (figure 3.28 Ba-Bc, Be-Bf). Here
again, adding a site specificity in terms of $p$ did not further increase the deviation
of the estimates from the model parameters (compared with q-specific sites, in red).

The same but amplified shifts in parameter estimation were revealed in the cumulative distributions obtained from the simulations of the complex binomial model in which $p$ and $q$ values were correlated across sites (in black). This observation, together with the resemblance in shape to sigmoids issued from $q$ estimation in the anti-correlated case (particularly figure 3.28 Be-h, in purple) and the consideration of the median values from the 3 groups of distributions discussed, would suggest that, under the conditions described, the definition of sites with specific quantal sizes ($q$) did induce these shifts in parameter estimation. Site specific probability of release ($p$) alone did not generate additional deviation consistently. Nevertheless, the correlation status between these two parameters across sites was seen to moderate or amplify the shift induced by specificity in quantal size alone, depending on whether this status was respectively negative or positive.

A satisfying explanation for the pattern of disruptions in parameter estimation, results from considering the known behaviour of binomial models with site specificity in probability of release. Release sites with low probability contribute less to the statistics even to the point of being invisible to fluctuation analysis methods (thus the estimates in our approach are termed "functional" and the number of release sites allowed to take rational values rather than strictly integer ones).

When the ranges of $p$ values across sites were restricted to biologically relevant values, parameter estimation was not disrupted by incorporating more complexity in the models. No specific additional deviation could be seen in the complex model in which $p$ and $q$ were uncorrelated across sites. In short, the respective contribution of sites with bigger or lower quantal sizes can be modulated through their specific probability of release, correspondingly
amplifying or moderating selectively this type of disruption in the statistics and consequently in parameter estimation. Hence variations in $p$ were only synergistic to those in $q$ for the disruption of parameter estimation.

The general direction of the shifts in parameter estimation suggested that specificity in $q$ produced the major effect since the sites with higher $q$ would more powerfully affect the statistics and that the fitting procedures would tend to provide an overestimation of $q$ (figure 3.28 B), and an underestimation of $n$ (figure 3.28 A).

The special situations displayed by the "Failure rate against M" method (figure 3.28 Ad, Ah, Bd and Bh) resulted primarily from the high level of imprecision and jitter in the failure rate statistics obtained in high $p$-conditions (Ah and Bh). Cumulative profiles that were not sigmoidal in shape were generated in these particular cases, hence the underlying distributions were not Gaussian in shape. On the other hand, in low $p$-conditions, application of this method led to clear sigmoid profiles for $q$ estimation (Bd) that were not reciprocated in $n$ estimation (Ad). Again, the control case from simple binomial model simulations reliably crossed half accumulation threshold at a median value equal to the original mean $q$ value. Interestingly the pattern of presumed shifts in $q$ estimation differed from the one common to the other 3 methods. Here, only the conjunction of $p$ and $q$ site specifications and further the strict character of the correlation between them (positive or negative, in black and purple respectively) would entrain a noticeable deviation (Bd). Moreover, the direction of the shifts from the appropriate basal condition (uncorrelated, in blue) was opposite between the two situations, leading to an underestimation of $q$ i.e. lower apparent mean $q$ in the anti-correlated case (Bd, in purple).
3.4.3.2 Statistical similarities in binomial models evaluations

To evaluate the relevance of the described deviation patterns in binomial parameter estimation (see section 3.4.3.1), statistical assessment was performed. Considering the non Gaussian shape of some of the distributions, notably that resulting from the “Failure rate against M” fitting method, a preliminary all to all evaluation of statistical differences was performed using 2-sample Kolmogorov-Smirnov (KS) test. This non-parametric test addresses the identity of two continuous distributions, by comparing the proportions of values from both distributions below every value along their whole range. The maximum difference between these is the test statistic over the hypothesis that the distributions are the same. Because the above procedure provides a 48 by 48 matrix (4 fitting methods applied to 6 models in 2 different p-ranges), the results are summarised as presented in figure 3.29.

For each pair of binomial models, the number of occurrences of non-rejection through the procedure is indicated and colour coded for significance level 1% and 5% within each two entry tables. This identified the models from which the distributions of estimates were usually found to be similar. As an example, the distributions of $n$ estimates obtained from the Simple binomial model and those obtained from the Complex model with $p$-specific sites were not statistically different in 19 cases out of 32 comparisons at the 5% level (figure 3.29 A1, below the identity diagonal), and still not in 15 cases at significance level 1% (above the identity diagonal). This can be interpreted as a confirmation of the low level of discrimination between statistics of samples from these two models by the fitting methods.

Consequently this supported the claim that within the limits imposed, estimation would not be impaired if such complexity was affecting neurotransmitter release at a neuronal connection. Actually in this case, the
Figure 3.29: Summary of statistical comparisons between distributions of parameter estimates from Monte Carlo simulations of six different binomial models with identical mean parameters. The number of times the distributions across Monte Carlo repetitions of binomial estimates for pairs of binomial models were not found different is indicated and colour coded in each table. This degree of similarity in parameter evaluation was assessed through Kolmogorov-Smirnov (K-S) non parametric test for \( n \) and \( q \) (respectively in A1 and A2), and Student's \( t \)-test (\( t \)-test, in B1 and B2). Because of systematic differences, complete similarity values would be 32 in A1 and B1, and 64 in A2 and B2.
difficulty in discriminating between the models was further evidenced for mean quantal size \((q)\) estimation, with 44 and 38 occurrences of statistical identity between distributions out of 64 comparisons at 5% and 1% significance level respectively (figure 3.29 A2).

This screening by KS test delineated two main groups of binomial models: Simple, Complex with \(p\)-specific sites and Complex with anti-correlated \(p\) and \(q\) site specificity models on one hand, and Complex with \(q\)-specific sites and Complex with uncorrelated \(p\) and \(q\) site specificity on the other hand (Figure 3.29 A). The same systematic procedure performed using Student's \(t\)-test led to a similar but more gradual picture of the inability to discriminate between models using the 4 fitting methods (Figure 3.29 B).

Overall the maximum shift in distribution of estimates as evaluated from the median values was 1.10 for estimated \(n\) and 0.12 for estimated \(q\). These represent less than 15% of their respective sought values. Since the differences in binomial parameters between different types of cortical connection (Brémaud et al., 2007) were larger than the differences that resulted from implementing more complex binomial models, these fitting methods were considered to provide useful comparative estimates and the simple binomial model to be a useful model of release in this system.

### 3.4.3.3 Functional estimates from smaller samples

Monte Carlo simulations of a complex binomial model were used to compare the profiles of 60 draw samples with the profiles of 300 draw samples. This approach is presented in figure 3.30, focussing on the complex binomial model in which individual \(p\) and \(q\) values were correlated across sites (see section 2.6.3).
Figure 3.30: Distributions of statistics from Monte Carlo simulations of a complex binomial models using different sample sizes. The four statistics of samples generated by Monte Carlo simulation of a complex binomial models (n=4, mean q=0.5), are plotted against their Mean amplitude: Coefficient of Variation (A), Failure rate (B), Variance (C) and the ratio of the Variance to the Mean amplitude (D). Each dot is the result of a 300 draws sample (black) or 60 draws sample (grey). At each of 10 p-conditions, the results from 100 samples are shown. The wider jitter along both dimensions of statistics from smaller samples leads to expect that the fitting methods would provide less accurate functional estimates when the sample size is reduced.
When the statistics from 100 simulations of this complex binomial model using a sample size of 300 draws (in black) were superimposed on their counterpart using a sample size of 60 draws (in grey), all relationships showed a complete overlap of the two distributions, that were centred around common average values. Not surprisingly, the smaller samples presented a larger horizontal and vertical spread of values. This predicts that the respective fitting methods provide less accurate estimates when the sample size is reduced.

The performance in parameter estimation of the fitting methods under different simulation conditions was evaluated. A measure of this performance called *Validity* was defined as the product of relative *Accuracy* and the relative *Precision* (figure 3.31 A) displayed by the distributions of each estimate (relative to the actual mean parameter) provided by a fitting method, averaged over all six binomial models, with different sample sizes (number of draws from which the statistics were calculated), different numbers of release sites, and a range of high or low mean probabilities of release. The results obtained from this approach are shown as bar plots in figure 3.31, B1 and B2.

The CV, Variance and Variance/M fitting methods (in blue, light blue and yellow respectively) displayed very similar profiles of Validity measure in either $n$ or $q$ estimation across all simulation configurations considered. In contrast, the Failure rate method (in red) generally displayed a specific profile in Validity, particularly for the estimation of the number of release sites (figure 3.31 B1).

In the simulation configuration studied in detail in the previous sections (from section 3.4.2 onwards), considering statistics from samples of 300 draws of a connection with 8 release sites, the CV, Variance and Variance/M methods all displayed similarly high values of the Validity measure. The Validity of these three methods in $n$ estimation was lower when they were applied to simulations using a range of lower probabilities of release (figure 3.31 B1). However in these
Figure 3.31: Validity of fitting methods in parameter estimation over all binomial models in different simulation configurations. A: The Validity measure of parameter estimation was defined as the product of the relative Accuracy (distance of the distribution median to the actual mean parameter) by the relative Precision (±2 standard deviation range, includes ~95% of normally distributed data), as illustrated in the graph and equation. B: The average Validity measure in n (B1) and mean q (B2) estimation of four fitting methods (see key) over all models are shown for different simulation conditions regarding sample size (300 or 60 draws), number of release sites (8 or 4) and probability of release range (High or Low). Some conditions were adverse to all methods in estimation of n, and the Failure rate method displayed a unique profile of dependency upon the simulated conditions.
two configurations their Validity in mean $q$ estimation was very similar (figure 3.31 B2).

The validity measure of the Failure rate method appeared negative in both configurations for the estimation of $n$, indicating that a large value in Precision was leading to a negative value in relative Precision (relative Accuracy is always positive as it sanctions a distance). The Validity in $q$ estimation of the Failure rate method appeared similarly impaired by large values of relative Precision in simulations using a range of high probabilities of release. However its Validity in $q$ estimation reached values similar to the three other methods for simulations using a range of low probabilities of release (figure 3.31 B2; comparing the two leftmost sets of bars). These results were therefore in accordance with the interpretations drawn from the cumulative distributions of estimates presented in section 3.4.3.1 and figure 3.28.

Similarly high values of Validity in both $n$ and $q$ estimation were displayed by the CV, Variance and Variance/M fitting methods for simulations of connections with 4 release sites that used sample sizes of 300 draws, indicating that these methods could discriminate between binomial models of connections with different number of release sites. In these configurations, the Failure rate method also displayed high values of Validity in $q$ estimation, and low but positive values in $n$ estimation. The estimates of $n$ provided by the Failure rate method when probability of release is low can represent a valuable independent assessment of such functional profiles thus complementing the other three methods.

High values of Validity in $n$ and/or $q$ estimation could be reached by all methods when applied to simulations that yielded statistics from samples of 60 draws (right hand side of dotted vertical line in figure 3.31 B1 and B2). However these values would still be lower than those obtained from larger samples, and a
range of low probability of release led specifically to very low Validity values in \( n \) estimation.

The Validity profile of the Failure rate method indicated little reliability in \( n \) estimation from smaller samples, notably due to negative values in relative Precision. The Validity of the Failure rate method in \( q \) estimation however, indicated performances similar to, or in one instance (4 sites with low release probabilities) better than the other three methods. This with the exception of the specific case of a large number of release sites and high probabilities of release. This confirmed that the failure rate method can be used as a valuable independent assessment of such functional profiles and complements the other three methods.
3.5 BINOMIAL PARAMETERS FOR LAMINAR CONNECTION CLASSES

Excitatory connections in different layers of the neocortex were compared with regard to the relationships between statistical measures (mean and variability) of the fluctuations in their EPSP amplitude.

3.5.1 Binomial parameter estimation in different cortical layers

Fluctuation analysis performed against the complex short term dynamics exhibited by their postsynaptic potentials provided a functional account of connections between pyramidal cells in different neocortical layers.

3.5.1.1 Analysis of a pyramid-pyramid connection in layer 3

The electrophysiological characteristics of a layer 3 connection between two pyramidal cells are shown in figure 3.32. Both the presynaptic and postsynaptic cells displayed an adapting firing pattern upon steady intracellular current injection, as is typical of many pyramidal cells in supragranular layers (figure 3.32 A1).

The shape of the average EPSP elicited in response to a single presynaptic action potential is shown in figure 3.32, A2. The peak amplitude of the average single spike EPSP was 1.42 mV, its 10-90 % rise time 0.80 ms and width at half amplitude 9.70 ms. The time course of the spike triggered membrane potential standard deviation followed closely the shape of the average single spike EPSP, indicating that the time of highest variability in the recorded membrane potential coincided for all release events.

Average traces of the 2\textsuperscript{nd} EPSP at different interspike intervals are shown colour coded according to their measured peak amplitude in figure 3.32, B1-B3.
Figure 3.32: Electrophysiological characteristics of a L3 pyramid-pyramid connection. A: The responses to current injections (A1) and average EPSP elicited by a single presynaptic action potential and superimposed standard deviation time course (A2) are shown (presynaptic in black, postsynaptic in red). Average single EPSP characteristics: peak amplitude, 1.42 mV; width at half amplitude, 9.70 ms; rise time, 0.80 ms. B: Average 2nd EPSPs, colour-coded for peak amplitude, over a range of interspike intervals recorded at three different resting membrane potentials -67 mV (B1), -69 mV (B2) and -79 mV (B3). C: Smoothed single sweep measures of 2nd, 3rd and 4th EPSPs amplitude recorded at a membrane potential of -67 mV, are plotted against preceding interspike interval (C). The dashed black line indicates the first EPSPs average amplitude.
The corresponding traces were collected at three postsynaptic resting membrane potentials: -67 mV (B1), -69 mV (B2) and -79 mV (B3). A decrease in the 2nd EPSP amplitude was apparent at the shortest interspike intervals, while all three amplitude scales indicate that it could also, at some longer intervals, be larger than the single spike EPSPs average amplitude.

This short lived depression of the 2nd EPSP amplitude at the shortest interspike intervals also appeared in the plot of smoothed single sweep measures of amplitude against interspike interval (Figure 3.32 C). The moving average of 2nd EPSP amplitude exhibited depression at the shortest interspike intervals (<10 ms), and matched the average amplitude of 1st EPSPs at intervals as short as 15 milliseconds following the first AP. Peaks and troughs in the smoothed amplitudes time course were seen over the range of longer interspike intervals. The moving averages of the 3rd and 4th EPSP amplitudes presented further depressed values over the range of interspike intervals recorded, as well as interruptions in the time course of recovery from depression.

Subsets of 1st EPSPs at different degrees of post-tetanic potentiation, and subsets of 2nd, 3rd and 4th EPSPs from the peaks and troughs of the smoothed amplitude time course were selected. The statistics from the corresponding single sweep measures of amplitude provided the CV^2 plot in figure 3.33, A. All points fell in the portion of the plot indicative of a mean amplitude larger than the amplitude of the non-potentiated 1st EPSP subset. Most points fell in the region I of the plot (see section 3.2.3.2) indicating a presynaptic origin for their differences relative to the subset of non-potentiated 1st EPSPs. The subset of potentiated 1st EPSPs, however, displayed an unchanged level of amplitude variability despite its larger mean amplitude. This last case being more consistent with a postsynaptic origin for the changes in amplitude, the subset of potentiated 1st EPSP was excluded from the ensuing estimation of binomial parameters procedure.
Figure 3.33: Fluctuation analysis estimating \( n \) and \( q \) applied to a layer 3 pyramid-pyramid connection. A: \( CV^2 \) plot of subsets of EPSPs amplitudes recorded at a resting membrane potential of \(-67\) mV. B: Curves illustrated were fit to all subsets of non-potentiated 1° EPSP and 2°, 3° and 4° EPSPs at different interspike intervals (see colour key in A). B1: Coefficient of Variation plotted against Mean amplitude (Equation 1). B2: Failure rate plotted against M (Equation 2). B3: Variance plotted against M (Equation 3). B4: Variance/M plotted against M (Equation 4). Estimates obtained for \( n, q \) and initial probability of release (\( p \)), and the corresponding coefficient of determination (\( r^2 \)) are given as inserts.
The results obtained in applying the four fitting methods (see section 3.2.3.3) to the statistics of the selected subsets of 1st to 4th EPSP amplitudes are shown in figure 3.33, B1-B4. Three methods indicated very similar values for the fitting parameters, the number of functional release sites (n) and mean quantal amplitude (q), with values of n ranging from 2.91 to 2.99, and values of q from 0.67 to 0.68 mV. The Variance against Mean amplitude (M) fit (figure 3.33 B3) was associated with a null coefficient of determination (see section 2.4.3).

The same fluctuation analysis performed on subsets of EPSPs selected from recordings of the same layer 3 connection, but at the more hyperpolarised postsynaptic membrane potential of -79 mV are shown in figure 3.34, together with the results obtained at -67 mV (in grey) as introduced in figure 3.33.

Subsets of the 1st EPSP at different degrees of post-tetanic potentiation and 2nd, 3rd and 4th EPSP were selected and the statistics from the corresponding single sweep measures of amplitude provided the CV^2 plot in figure 3.34, A. Most points fell in the portion of the plot indicative of a mean amplitude larger than the amplitude of the non-potentiated 1st EPSP subset, with the exception of one subset of 4th EPSPs which displayed a slightly smaller mean amplitude. All points, notably the subset of potentiated 1st EPSPs (compare with figure 3.33 A), fell in the region I of the plot (see section 3.2.3.2) indicating a presynaptic origin for their differences relative to the subset of non-potentiated 1st EPSPs. When the subset of 1st EPSPs recorded at the more depolarised membrane potential of -67 mV was plotted on this CV^2 graph (figure 3.34 A, grey dot), it fell in the region II, indicating a postsynaptic origin for the differences in amplitude fluctuation between these two conditions.

The results of applying the four fitting methods (see section 3.2.3.3) to the statistics of the selected subsets of 1st to 4th EPSP amplitudes are shown in figure 3.34, B1-B4. Two fitting methods were associated with non-null values of
Figure 3.34: Estimating $n$ and $q$ for a layer 3 pyramid-pyramid connection at two different postsynaptic membrane potentials. **A:** CV$^2$ plot of subsets of EPSPs amplitudes. **B:** The curves illustrated were fit to all points including subsets of 1$^{st}$ EPSPs exhibiting different degrees of post-tetanic potentiation and subsets of 2$^{nd}$, 3$^{rd}$ and 4$^{th}$ EPSPs at different interspike intervals (see colour key in A) recorded at a postsynaptic membrane potential of -79mV or -67mV (in grey, from figure 3.22). **B1:** CV plotted against M (Equation 1). **B2:** F plotted against M (Equation 2). **B3:** Variance plotted against M (Equation 3). **B4:** Variance/M plotted against M (Equation 4). Estimates obtained for $n$, $q$ and initial probability of release ($p$), and the corresponding coefficient of determination ($r^2$) are given as inserts.
the coefficient of determination (figure 3.34 B1 and B2). These indicated \( n \) values of 4.64 and 3.14, and \( q \) values of 0.73 and 1.04 mV.

The figures 3.34, B1-B2, also show superimposed (in grey) the points and fits obtained from the subsets of EPSPs collected at the more depolarised resting membrane potential of -67 mV for comparison. The Failure rate against M method (figure 3.34 B2) displayed a graphical pattern and set of binomial estimates consistent with a change in mean quantal amplitude between the two conditions (see section 2.4.3 and figure 2.4), with well separated more or less parallel fitted curves.

3.5.1.2 Analysis of connections recorded in layer 2 to 6

The process and outcomes of applying fluctuation analysis to connections between pyramidal cells in layers 2, 3, 4, 5 and 6 recorded during this study are detailed in figures 3.35 to 3.46 (pages 132 - 143).

For some connections, the electrophysiological characteristics of the recorded cells and the short term synaptic dynamics were depicted in more detail. These included the firing patterns upon steady injection of current, the average shape of EPSPs triggered by single presynaptic action potentials (see section 3.1.3) and corresponding standard deviation time course, colour coded average traces of the 2\(^{nd}\) EPSPs at different interspike intervals, moving average of EPSP amplitude against preceding ISIs and/or plots of 2\(^{nd}\), 3\(^{rd}\) and later EPSP amplitudes against combinations of preceding synaptic activity characteristics (see section 3.2).
In all cases, over the course of recovery, peaks and troughs were apparent in the moving average plots. At strongly depressing synapses (figures 3.40, 3.41, 3.43, 3.45) the average amplitude of 2nd, 3rd and later EPSPs did not reach the average amplitude of the 1st EPSPs at the longest interspike intervals considered. When those time courses could be fitted with mono-exponential functions, the best fits reached a plateau at the longest interspike intervals that corresponded to a residual loss of 10 to 15% compared to the average 1st EPSP amplitude. This late phase of depression, the resorption of which our rate of stimulation (0.33 Hz) seemingly allowed since the amplitude of 1st EPSPs was stable across all the recordings analysed, is a common observation at neocortical connections.

At a layer 4 connection (figure 3.39, C1-C4), the emergence of bands of amplitudes (i.e. colour) similarly oriented perpendicularly to the interspike interval axis and parallel to the preceding EPSP amplitude axis, was visible in both the C1 and C4 plots, indicating a stronger dependency of EPSP amplitude upon the preceding interspike interval than upon the preceding EPSP amplitude. No preferential banding was visible in similarly constructed two-dimensional interpolation graphs, when variables of the same kind were considered (figure 3.39 C2 and C3). Rather, pockets of higher or lower EPSP amplitudes were visible for certain combinations of preceding interspike intervals or preceding EPSP amplitudes.

In all cases fitting procedures were performed on subsets of EPSP amplitudes that fell in the region I of the CV^2 plots, indicative of a presynaptic origin for their differences relative to the reference subset (non potentiated 1st EPSPs).
The six connections recorded in layer 2 and 3 were predicted to be using between 2 and 6 release sites. The range of quantal amplitude estimates for these connections laid between 0.21 and 0.75 mV. However, only the connections between one pair of layer 3 pyramidal cells that were reciprocally connected (figures 3.36 and 3.37) were predicted to be using quantal amplitudes smaller than 0.3 mV. The lower bound of the range of $q$ estimates for all other layer 3 connections was then closer to 0.4 mV. The associated estimates of release probability ranged between 0.44 and 0.94.

Similar numbers of functional release sites and mean release probability were obtained at two connections recorded in layer 4 (figures 3.11 and 3.40), together with quantal amplitude estimates that were lower than those obtained at most layer 2/3 connections.

Both connections recorded in layer 5 were estimated to be using a large number of release sites (between 6 and 12), similar quantal amplitudes (0.31-0.34 mV), and a high release probability (>0.7).

The morphological features of a pyramid-pyramid connection in layer 6 are shown in figure 3.44. Both cell bodies were located in upper layer 6. Both cells had an apical dendrite extending vertically from the cell body through layer 5 and up to lower layer 4, with extensive oblique arborisation in the former and no further branching in the latter. The postsynaptic cell displayed a more prominent web of basal dendrites extending radially from its cell body (in blue) that was mostly confined to its layer of origin. The respective axonal arbours also displayed very distinct features. The presynaptic cell axonal arbor (in orange) gave rise to only few collaterals that extended with little further branching mainly in layer 6 and 5. In contrast, the postsynaptic cell axonal arbor (in purple) displayed a prominent corticofugal projection extending vertically directly
through lower layer 6 towards the underlying white matter and could be traced there on a more oblique course. From the initial portion of this axonal projection, few collaterals branched at nearly right angles invading layer 6 laterally in the tangential plane. From these, further branching gave rise to extensions more vertically oriented, that extended towards the neighbouring layer 5, one of which reached right through to lower layer 4 with little further intermediate branching, and another invaded layer 5 tangentially.

The fluctuations in EPSP amplitude recorded at this pyramid-pyramid layer 6 connection, appeared best described by assuming a large number of release sites (~14), a quantal amplitude close to 0.3 mV and a low probability of release (<0.27). Despite the large number and range of amplitude subsets that could be selected from this particular data set, and the apparent good performance of some of the fitting methods, the proposed functional profiles, in requiring a low initial release probability, appear at odds with the strongly depressing dynamic behaviour (figure 3.45). Possible explanations for the inadequacy of the model in this particular case include a mixed contribution of changes in $n$ as well as $p$ to the short term depression (most subsets closer to the identity line than the vertical iso-amplitude line in figure 3.46 A) and/or a high incidence of presynaptic branch point failures of the action potential propagation (failure rate offset even for the subsets with the highest mean amplitudes in figure 3.46 B2).
Figure 3.35: Fluctuation analysis estimating $n$ and $q$ applied to a layer 2 pyramid-pyramid connection. A: CV$^2$ plot of subsets of EPSPs amplitudes, the morphology and time courses of successive EPSPs amplitude were presented in figure 3.9. B: The curves illustrated were fit to all points including subsets of 1st EPSPs exhibiting different degrees of post-tetanic potentiation and subsets of 2nd, 3rd and 4th EPSPs at different interspike intervals (see colour key). B1: Coefficient of Variation plotted against Mean amplitude (Equation 1). B2: Failure rate against M (Equation 2). B3: Variance against M (Equation 3). B4: Variance/M against M (Equation 4). Estimates obtained for $n$, $q$ and initial probability of release ($p$), and the corresponding coefficient of determination ($r^2$) are given as inserts.
Figure 3.36: Fluctuation analysis estimating $n$ and $q$ applied to a depressing layer 3 pyramid-pyramid connection. A: CV$^2$ plot of subsets of EPSPs amplitudes. B: Curves illustrated were fit to all subsets of $1^{st}$, $2^{nd}$, $3^{rd}$ and $4^{th}$ EPSPs (see colour key). B1: Coefficient of Variation plotted against Mean amplitude (Equation 1). B2: Failure rate plotted against $M$ (Equation 2). B3: Variance plotted against $M$ (Equation 3). B4: Variance/M plotted against $M$ (Equation 4). Estimates obtained for $n$, $q$ and initial probability of release ($p$), and the corresponding coefficient of determination ($r^2$) are given as inserts. Note: the cells were reciprocally connected (analysis of reciprocal connection in figure 3.25).
Figure 3.37: Fluctuation analysis estimating n and q applied to a depressing layer 3 pyramid-pyramid connection. A: CV$^2$ plot of subsets of EPSPs amplitudes. B: Curves illustrated were fit to all points including subsets of 1$^\text{st}$ EPSPs exhibiting different degrees of post-tetanic potentiation and selected subsets of 2$^\text{nd}$, 3$^\text{rd}$ and 4$^\text{th}$ EPSPs (see colour key in A). B1: Coefficient of Variation plotted against Mean amplitude (Equation 1). B2: Failure rate against M (Equation 2). B3: Variance against M (Equation 3). B4: Variance/M against M (Equation 4). Estimates obtained for n, q and initial probability of release (p), and the corresponding coefficient of determination ($r^2$) are given as inserts. Note: the cells were reciprocally connected (analysis of reciprocal connection in figure 3.24).
Figure 3.38: Fluctuation analysis estimating $n$ and $q$ applied to a depressing layer 3 pyramid-pyramid connection. A: $CV^2$ plot of subsets of EPSPs amplitudes. B: The curves illustrated were fit to all points including subsets of 1st EPSPs exhibiting different degrees of post-tetanic potentiation and subsets of 2nd, 3rd and 4th EPSPs at different interspike intervals (see colour key in C1). B1: Coefficient of Variation plotted against Mean amplitude (Equation 1). B2: Failure rate plotted against $M$ (Equation 2). B3: Variance plotted against $M$ (Equation 3). B4: Variance/M plotted against $M$ (Equation 4). Estimates obtained for $n$, $q$ and initial probability of release ($p$), and the corresponding coefficient of determination ($r^2$) are given as inserts.
Figure 3.39: Electrophysiological characteristics of a strongly depressing L4 pyramid-pyramid connection. A: The shape of the average EPSP elicited by a single presynaptic action potential was unaffected by presynaptic spikelets (presynaptic, top traces; postsynaptic bottom traces). B: Average single EPSP characteristics: peak amplitude 1.98 mV, width at half amplitude 14.5 ms, rise time 1.90 ms. Average 2nd EPSPs colour-coded for peak amplitude over a range of interspike intervals are shown. C: 2-dimensional plots of the 2nd (C1) and 3rd (C2-C4) EPSP amplitude measures (white dot) against preceding interspike interval and/or preceding EPSP amplitude maps complex synaptic dynamics.
Figure 3.40: Fluctuation analysis estimating $n$ and $q$ applied to a strongly depressing layer 4 pyramid-pyramid connection. A: Smoothed 2nd and 3rd EPSPs amplitudes plotted against preceding interspike interval. B: CV² plot of subsets of EPSPs amplitudes. C: The curves illustrated were fit to all points including subsets of 1st EPSPs exhibiting different degrees of potentiation and subsets of 2nd to 4th EPSPs (colour key in C1). C1: Coefficient of Variation plotted against Mean amplitude (Equation 1). C2: Failure rate plotted against M (Equation 2). C3: Variance plotted against M (Equation 3). C4: Variance/M plotted against M (Equation 4). Estimates obtained for $n$, $q$ and initial probability of release ($p$), and the corresponding coefficient of determination ($r^2$) are given as inserts.
Figure 3.41: Electrophysiological characteristics of a strongly depressing L5 pyramid-pyramid connection. A: The shape of the average EPSP (orange) elicited by a single presynaptic action potential (black) and the time course of the standard deviation (grey) are shown superimposed. Single EPSP characteristics: peak amplitude 2.00 mV, width at half amplitude 17.9 ms, rise time 2.20 ms. B: The shape of the average single EPSP (-80mV, orange) changed when recorded at a more hyperpolarised membrane potential (-90mV, red). The time course of the difference between the two traces is presented in purple. C: Some degree of post-tetanic potentiation was apparent, Kolmogorov-Smirnov test, ** p<0.01, subsets size indicated inside the bars, error bars show the standard deviation. D: Smoothed single sweep measures of 2nd, 3rd and 4th EPSPs amplitude (see colour key) plotted against preceding interspike interval. The horizontal dashed black line indicates the average amplitude of non potentiated first EPSPs. Dotted grey line indicates a monoeponential fit of the recovery from depression for the 2nd EPSP. Time constant 20 ms, coefficient of determination 0.94.
Figure 3.42: Fluctuation analysis estimating $n$ and $q$ applied to a strongly depressing layer 5 pyramid-pyramid connection. A: CV² plot of subsets of EPSPs amplitudes. B: The curves illustrated were fit to all points including subsets of 1ˢᵗ EPSPs exhibiting different degrees of post-tetanic potentiation and subsets of 2ⁿᵈ, 3ⁿᵈ and 4ⁿᵗʰ EPSPs at different interspike intervals (see colour key A). B1: Coefficient of Variation plotted against Mean amplitude (Equation 1). B2: Failure rate plotted against Mean amplitude (Equation 2). B3: Variance plotted against Mean amplitude (Equation 3). B4: Variance/Mean amplitude plotted against Mean amplitude (Equation 4). Estimates obtained for $n$, $q$ and initial probability of release ($p$), and the corresponding coefficient of determination ($r^2$) are given as inserts.
Figure 3.43: Fluctuation analysis estimating n and q applied to a depressing layer 5 pyramid-pyramid connection. A: Smoothed time course of 2nd to 4th EPSPs amplitude as a function of preceding interspike interval. B: CV² plot of subsets of EPSPs amplitudes. C: Curves illustrated were fit to all subsets of 1st, 2nd, 3rd and 4th EPSPs (see colour key in C1). C1: Coefficient of Variation plotted against Mean amplitude (Equation 1). C2: Failure rate plotted against Mean amplitude (Equation 2). C3: Variance plotted against Mean amplitude (Equation 3). C4: Variance/Mean amplitude plotted against Mean amplitude (Equation 4). Estimates obtained for n, q and initial probability of release (p), and the corresponding coefficient of determination (r²) are given as inserts.
Figure 3.44: Morphological and electrophysiological characteristics of a synaptic connection between two pyramidal cells in layer 6. Reconstruction reveals the laminar location and morphological features of the recorded cells. Cell body and dendritic tree of the presynaptic cell in red, axonal arbour in orange, postsynaptic cell respectively in blue and purple. Responses to intracellular current injections are shown with colours matching the cell bodies. The average EPSP (blue) and superimposed standard deviation time course (grey) in response to a single presynaptic action potential (red) are shown in the bottom left corner. Bottom right traces present the average EPSPs (blue) triggered by successive action potentials (red) in presynaptic trains.
Figure 3.45: Time course of recovery from depression of EPSPs triggered by successive presynaptic action potentials at a strongly depressing layer 6 pyramid-pyramid connection. Smoothed single sweep measures of amplitude are plotted against the preceding interspike interval, for the 2\textsuperscript{nd} (A), 3\textsuperscript{rd} (B), 4\textsuperscript{th} (C) and 5\textsuperscript{th} (D) EPSPs from the layer 6 connection introduced in figure 3.33. The horizontal dashed black line indicates the average amplitude of non potentiated first EPSPs. Dotted grey lines indicate monoexponential fits to the recovery from depression for the 2\textsuperscript{nd} and 3\textsuperscript{rd} EPSP. Coefficients of determination respectively 0.60 and 0.70, time constants 45 and 44 ms with different offsets. The 2\textsuperscript{nd}, 3\textsuperscript{rd} and 5\textsuperscript{th} EPSP smoothed time courses displayed pronounced interruptions in the recovery from depression, that can be used to define consistent subsets of amplitudes for the fluctuation analysis.
Figure 3.46: Fluctuation analysis estimating $n$ and $q$ applied to a strongly depressing layer 6 pyramid-pyramid connection. A: $CV^2$ plot of subsets of EPSPs amplitudes. B: Curves illustrated were fit to all points including subsets of 1st EPSPs exhibiting different degrees of post-tetanic potentiation and subsets of 2nd to 5th EPSPs at different interspike intervals (see colour key A). B1: Coefficient of Variation plotted against Mean amplitude (Equation 1). B2: Failure rate plotted against Mean amplitude (Equation 2). B3: Variance plotted against Mean amplitude (Equation 3). B4: Variance/Mean amplitude plotted against Mean amplitude (Equation 4). Estimates obtained for $n$, $q$ and initial probability of release ($p$), and the corresponding coefficient of determination ($r^2$) are given as inserts.
3.5.1.3 Analysis performed on previously recorded connections

Fluctuation analysis was also applied to a larger population of previously recorded connections that presented suitable conditions in signal stability (see sections 2.4.1 and 3.2.1).

In figure 3.47, data obtained from a depressing layer 4 pyramid–pyramid connection (figure 3.47 A) and a facilitating layer 6 pyramid to layer 5 bitufted interneurone connection (figure 3.47 B) are plotted. Although there is scatter, measurements obtained from first, second, and, in figure 3.47 A, third, fourth, and fifth EPSPs in trains could be described by the four relationships, and the estimates of \( n \) and \( q \) obtained with the four methods were similar.

When the Coefficient of Variation, Failure rate, Variance, or Variance/M are plotted against Mean amplitude (M), the binomial model predicts that changes in \( p \) alone will shift points along the lines given by equations 1–4 (section 2.4.3 and figure 2.4).

Two pyramid–pyramid pairs (figure 3.48 A) and 3 pyramid–interneurone pairs (figure 3.48 B) were challenged with \( \omega \)-Conotoxin GVIA and one pyramid–pyramid connection with \( \omega \)-Agatoxin IVA. Essentially similar results were obtained in all experiments with presynaptic Ca\(^{2+} \) channel blockers. Data subsets corresponding to first EPSPs expressing different levels of posttetanic potentiation and to second, third, and fourth EPSPs at different interspike intervals were obtained. Control data and test data were plotted and fitted separately. Estimates of \( n \) and \( q \) were similar with all four methods and for control and \( \omega \)-Conotoxin GVIA / \( \omega \)-Agatoxin IVA data, indicating that the differences between the two conditions were predicted to arise from a decrease in the remaining binomial parameter, mean release probability.
Figure 3.47: The 4 methods of estimating $n$ and $q$ applied to a depressing layer 4 pyramid-pyramid (A) and a facilitating layer 6 pyramid to layer 5 bitufted interneurone connection (B). The curves illustrated were fit to all points including subsets of 1st EPSPs exhibiting different degrees of post-tetanic potentiation and subsets of 2nd, 3rd, 4th and 5th EPSPs at different interspike intervals (see key A1). A1, B1: Coefficient of Variation plotted against Mean amplitude (Equation 1). A2, B2: Failure rate plotted against Mean amplitude (Equation 2). A3, B3: Variance plotted against Mean amplitude (Equation 3). A4, B4: Variance/Mean amplitude plotted against Mean amplitude (Equation 4). Estimates obtained for $n$ and $q$ are given as inserts. For these plots $r^2$ (coefficient of determination) was 0.85 in A1, 0.91 in A2, 0.62 in A3, 0.60 in A4, 0.87 in B1, 0.96 in B2, 0.65 in B3 and 0.46 in B4.
Figure 3.48: Changes in binomial estimates induced by a pharmacological challenge. A layer 3 depressing pyramid-pyramid connection (A) and a layer 3 depressing pyramid to fast spiking, multipolar interneurone connection (B). These pairs were first recorded under control conditions (filled circles), then after addition of ω-conotoxin GVIA (open). Following addition of this N-type Ca2+ channel blocker EPSPs decreased in average amplitude (M) and the proportion of failures (F) increased. **A1, B1**: EPSP Coefficient of Variation plotted again Mean Amplitude. **A2, B2**: Failure rate plotted against Mean Amplitude. Control and ω-conotoxin GVIA data were fit separately. Estimates for n and q are given as inserts. For these plots r² (coefficient of determination) was A1 control 0.83, conotoxin 0.92, A2 control 0.90, conotoxin 0.95, B1 control 0.75, conotoxin 0.93, B2 control 0.85, conotoxin 0.96.
3.5.2 Binomial estimates for cortical connection classes

From a larger population, paired recordings were selected for further analysis. Attention focussed on longer paired recordings containing multiple data subsets and included 69 connections between excitatory cells, 16 in cat and 53 in rat, and 30 connections from pyramids to interneurones, 13 in cat and 17 in rat. In all, 949 data subsets and over 50,000 EPSPs measured individually were included.

3.5.2.1 Functional landscape of cortical connectivity

Clear differences in the regions of parameter space occupied by pyramid–pyramid connections in different layers were apparent when the Coefficient of Variation, Failure rate, and Variance/M were plotted against M (figure 3.49).

In rat, intralaminar layer 3 and layer 5 connections occupied regions indicative of a higher $q$ than connections within layer 4 or from layer 4 to layer 3. Connections in layer 6 occupied intermediate positions (figure 3.49 A).

In cat, connections from layer 6 pyramids again occupied intermediate regions, but layer 3 pairs appeared to exhibit a smaller $q$ and layer 4 a larger $q$ in this species (figure 3.49 B).

Within layer 6, corticocortical pyramidal outputs exhibit depression, whereas those of corticothalamic pyramids facilitate (Mercer et al., 2005; West et al., 2006). Figure 3.50 shows that connections made by these two classes of pyramids occupied opposite ends of their parameter space albeit with some overlap. Fluctuation analysis provided a functional account for these differences whereby the depressing outputs of layer 6 corticocortical pyramids were predicted to use a higher $p$ at low firing frequencies than facilitating corticothalamic pyramid outputs. The estimates of $q$ obtained with the four methods were similar for these connections ($P>0.1$, Student's unpaired $t$-test),
Figure 3.49: Cortical connections in different layers occupy different regions of the parameter space. Comparison of connections between pyramids in different layers and two species, rat (A) and cat (B). Measurements from data subsets are plotted. Colour codes the layer in which the pre- and postsynaptic neurons lay (key B2). **A1, B1:** EPSP Coefficient of Variation plotted against Mean amplitude. **A2, B2:** Failure rate against Mean amplitude. **A3, B3:** Variance/Mean amplitude against Mean amplitude. In rat (A1-3), points from layer 3 (red) and layer 5 pairs (yellow) lie in a region of parameter space indicative of a larger $q$ than those from layer 4 (green). Points from layer 6 (blue) lie between. In cat (B1-3) points from connections involving layer 4 cells (L4 to L4, L4 to L3 and L5 to L4) occupy regions of parameters space indicative of a larger $q$ than connections between layer 3 pyramids, with layer 6 between.
Figure 3.50: Similar quantal sizes were estimated for layer 6 connection classes occupying opposite ends of their parameter space. Intra-laminar connections made by presynaptic cortico-thalamic pyramids with postsynaptic layer 6 pyramids (closed triangles) compared with intra-laminar connections made by presynaptic cortico-cortical pyramids (open circles). Cortico-cortical pairs for which only one data set was available are indicated by half-filled circles. Coefficient of Variation is plotted against Mean amplitude. The separate regions of parameter space occupied and mean estimates of $n$ and $q$ (inserts) demonstrate that while $q$ is similar for both populations ($P>0.5$), estimates of $n$ and of $p$ for low frequency single spike EPSPs are larger for cortico-cortical connections than for connections made by cortico-thalamic axons ($P<0.001$).
but estimates of $n$ and $p$ (assuming $p = M/nq$) for corticocortical outputs were twice those for corticothalamic outputs ($P < 0.05$). Within layer 6 then, were two classes of connections where the estimates of $q$ were similar but where differences in $n$ and $p$ were predicted to result in significant differences in $M$.

### 3.5.2.2 Classes of excitatory connections onto interneurones

Excitatory inputs onto interneurones differed from those onto pyramidal cells. In plots of the Coefficient of Variation against $M$ (figure 3.51), points for facilitating inputs onto bitufted interneurones with broad action potentials (width at half amplitude $> 0.3$ ms) and adapting or burst firing characteristics occupied regions of parameter space that were largely separate from connections onto pyramids (figure 3.51 A). This was also true for the depressing inputs onto layer 3 multipolar interneurones with narrow action potentials (width at half-amplitude $\leq 0.3$ ms) plotted in figure 3.51, B.

### 3.5.2.3 Associated binomial estimates

Plots of Coefficient of Variation, Failure rate, Variance or Variance/$M$ against Mean amplitude ($M$) were fit with the four relations for each connection. For the majority, correlations for fits by equations 1–4 were above 0.8. For each parameter estimate, coefficients of variation (CVs) were calculated. For estimates of $q$, CVs were lower (mean 0.18, 0.24, 0.18, 0.19, $P < 0.001$ Student's unpaired $t$-test) than CVs for estimates of $n$ (0.45, 0.99, 0.99, 0.39).

Correlations between estimates of $n$ and between estimates of $q$ obtained with methods 1, 3, and 4 were strong (correlations $> 0.91$), had slopes close to unity (0.89–1.08), and intercepts close to zero (0.01–0.69 for $n$ and -0.04–0.06 for $q$; figure 3.52). Correlations between estimates of $n$ and $q$ obtained with
Figure 3.51: Points obtained from connections onto interneurones occupy a distinct parameter space. Coefficient of Variation plotted against Mean amplitude for pyramidal inputs onto bitufted, dendrite-targeting interneurones (A) and fast spiking, multipolar parvalbumin immuno-positive interneurons (B). Closed symbols indicate rat and open symbols cat data, layers are colour coded (key in A). Pyramidal data from figure 3.47 A1, and figure 3.48 B1 are shown in grey for comparison. Almost all bitufted and multipolar interneurone data from layer 3 fall outside the parameter space occupied by pyramid-pyramid connections, indicating that these connections display different binomial parameters.
Figure 3.52: Strong correlations between the values of the parameters $n$ and $q$ obtained using the 4 binomial model-based methods. From original data subsets, EPSP coefficient of variation (CV), proportion of failures ($F$), variance ($V$) and variance divided by mean EPSP amplitude ($V/M$) were calculated and plotted against $M$ for each paired recording. These 4 plots were then fit with Equations 1-4 given in the Methods to obtain estimates of $n$ (A) and $q$ (B) for each of the recorded pairs that are plotted here (see figures 3.10 and 3.11 for an example). The correlations illustrated here demonstrate that the 4 methods produce very similar estimates for the binomial parameters.
method 2 (failure rate) and those obtained with methods 1, 3, and 4 were less strong (0.7–0.87), but slopes and intercepts were similar.

The correlation between measures of the paired pulse ratios (PPR) and the estimated initial release probabilities is shown in figure 3.53. Most values of estimated release probability at depressing connections (PPR<1) were between 0.25 and 1.00, while most facilitating connections (PPR>1) estimates were between 0.02 and 0.70. The negative correlation between estimates for $p$ (consecutive to $n$ and $q$ estimation) for low-frequency single-spike EPSPs and the paired-pulse ratio (correlation>0.80), indicated that the estimates of $n$ and $q$ obtained provided a useful comparison.

The order apparent in figure 3.49 was paralleled by the estimates of $q$ for individual connections provided in table 3.2. In rat, EPSPs generated by layer 6 corticothalamic pyramids were significantly smaller than those of other pyramid–pyramid connections ($P<0.02$, Student's unpaired $t$-test). Layer 3 pyramid–pyramid EPSPs in rat were larger than those in layer 4 ($P<0.01$) but similar in size to those in layer 5 and to the outputs of layer 6 corticocortical pyramids ($P<0.29$). No significant differences in $M$ were apparent among the populations of cat pyramid–pyramid connections and, as reported (Thomson et al., 2002; Mercer et al., 2005), no significant differences in $M$ were apparent between rat and cat data.

Mean estimates of $q$ for all pyramid–pyramid connections were smaller than for pyramid–interneurone connections ($P<0.01$, Student's unpaired $t$-test). Mean estimates of $p$ for depressing inputs onto fast spiking interneurones were similar to those for pyramid–pyramid connections, but for facilitating inputs onto bitufted interneurones, estimates of $p$ were significantly lower ($P<0.01$, table 3.2).
Figure 3.53: Correlation between measures of the paired pulse ratios and estimated release probability for cortical connections. Paired pulse ratio (the amplitude of the averaged 2nd EPSP divided by the amplitude of the 1st EPSP) was calculated for 2nd EPSPs at different interspike intervals (different symbols, see key) and plotted against estimated p for each connection. Estimates of p were obtained by dividing the mean EPSP amplitude at low firing rates by estimated n and q. These relations were fit with a simple power relation and the curves fit to 10-15 and 20-30msec interval data are shown (correlation > 0.8).
### Table 3.2: Comparison of binomial estimates between neocortical connection classes.

Mean amplitudes ($M$) of single spike EPSPs at low firing rates and estimated $n$, $q$, and $p$ for the major populations of pyramid-pyramid and pyramid-interneurone connections analysed. Facilitating outputs of cortico-thalamic pyramids were excluded from the pooled data for all layers in the lower part of the table. Whether these parameters were similar for different types of connections was tested with ANOVA and Student’s unpaired t-tests. ANOVA indicated that there were differences in $M$, $n$, $q$, and $p$ amongst pyramid-pyramid populations from rat cortex, between pyramid-pyramid and pyramid-interneurone connections in rat and cat and in $p$ and $q$ for cat cortex ($P<0.05$). Significant differences suggested by these comparisons are indicated by the labels in the table. $n$ - number of contributory release sites; $q$ - quantal amplitude; $p$ release probability. L3 - layer 3; L4-L3 - layer 4 to layer 3; L6CC - layer 6 cortico-cortical; L6CT - layer 6 cortico-thalamic; PMu - pyramid to multipolar interneurone; PBi - pyramid to bitufted interneurone; PP - pyramid to pyramid.

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<th>$q \pm SD$</th>
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<td>L3-L3 (n=8)</td>
<td>1.53 ± 0.29</td>
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<td>0.99 ± 0.46</td>
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<td>L5-L5 (n=6)</td>
<td>1.41 ± 0.74</td>
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<td>CT</td>
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<td>L6CC-L6 (n=4)</td>
<td>1.12 ± 0.58</td>
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<td>L6CT-L6 (n=4)</td>
<td>0.27 ± 0.17</td>
<td>2.7 ± 1.3</td>
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<td>L3, L4, L5</td>
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<td><strong>Cat Pyramid-Pyramid</strong></td>
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<td>L3-L3 (n=5)</td>
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<td>L4-L4 (n=5)</td>
<td>1.61 ± 1.03</td>
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<td>L6CC-L6 (n=3)</td>
<td>0.92 ± 0.35</td>
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<td>L4-L3 (n=4)</td>
<td>0.44 ± 0.50</td>
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<td>All PP (n=32)</td>
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<td>PMu (n=6)</td>
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<td>6.43 ± 2.33</td>
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<td>0.63 ± 0.25</td>
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<tr>
<td>Pbi (n=7)</td>
<td>0.52 ± 0.19</td>
<td>5.76 ± 3.90</td>
<td>0.67 ± 0.19</td>
<td>0.24 ± 0.17</td>
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<td>Pmu, PP</td>
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<td><strong>Cat Pyramid-Pyramid compared with Pyramid-Interneuron</strong></td>
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<tr>
<td>All PP (n=17)</td>
<td>1.22 ± 0.74</td>
<td>11.9 ± 8.03</td>
<td>0.28 ± 0.23</td>
<td>0.70 ± 0.21</td>
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<td>Pbi, Pmu</td>
<td>Pbi, Pmu</td>
<td>Pbi</td>
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<tr>
<td>PMu (N=6)</td>
<td>1.43 ± 1.46</td>
<td>7.64 ± 5.85</td>
<td>0.49 ± 0.23</td>
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<td>Pbi, PP</td>
<td>Pbi</td>
<td>Pbi</td>
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<tr>
<td>Pbi (n=3)</td>
<td>0.53 ± 0.08</td>
<td>2.74 ± 1.05</td>
<td>0.99 ± 0.31</td>
<td>0.28 ± 0.17</td>
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Graphical illustration of the preferential parameter spaces predicted for the connection classes identified in table 3.2 is provided in figure 3.54. In rat, estimates of $q$ for layer 3 pyramid-pyramid connections and for connections between layer 5 pyramids were larger than for connections between layer 4 pyramids (figure 3.54 A). The estimates for layer 6 cortico-cortical pyramid outputs fell in an intermediate position. These differences in the predicted mean quantal amplitude arose over a similarly wide range of estimates for $n$. Estimates of $q$ and $n$ for the outputs of cortico-thalamic pyramids placed these connections in a distinct region of the parameter space corresponding to lower values of $n$ combined with intermediate values of $q$. In cat, estimates of $q$ were typically smaller for connections in layer 3 than elsewhere (figure 3.54 B).

The predicted largest mean quantal amplitude at layer 3 pyramid-interneurone connections of all classes for comparable values of functional number of release sites can be seen in figure 3.54, C and D.

That further differences in $n$ might appear with larger samples was indicated by comparison of the slopes of linear fits to Variance/M against M plots of population data in which points that could not be used for parameter estimates (because data subsets were too few or too consistent in M) could be included. The outcome paralleled conclusions drawn from parameter estimates but suggested additional differences as 13:22 comparisons of pyramid–pyramid connections in rat and 5:9 in cat, and the majority of same-layer comparisons between pyramid–pyramid and pyramid–interneurone connections resulted in rejection of the hypothesis that the two populations fell on the same line at the 95–99% confidence level.
Figure 3.54: Estimates of $q$ obtained using the four methods plotted against estimates of $n$. Symbols indicate the method and colours the type of connection (see keys). In A (rat) and B (cat), pyramid-pyramid connections in different layers are compared. In C and D, pyramidal inputs onto two broad classes of interneurones are compared with pyramid-pyramid connections (grey circles). Open symbols indicate cat and closed symbols rat data. Dotted lines outline populations of connections. **A**: In rat, estimates of $q$ for layer 3 pyramid-pyramid connections and for connections between layer 5 pyramids are larger than for connections between layer 4 pyramids, with estimates for layer 6 cortico-cortical pyramid outputs lying between. This despite a similarly wide range of estimates for $n$. Estimates of $q$ and $n$ for the outputs of cortico-thalamic pyramids placed these connections in a distinct region of parameter space corresponding to lower values of $n$ with intermediate values of $q$. **B**: In cat, estimates of $q$ were typically smaller for connections from pyramids to interneurones there may also be differences between layers.
3.5.3 Comparative landscape in rat neocortex

When the Coefficient of Variation, Failure rate, Variance, and Variance/M were plotted against the Mean amplitude (M) for the recorded connections described in sections 3.5.1.1 to 3.5.1.4 (figure 3.55), the plots displayed features similar to their counterpart established for the larger population of rat cortical connections (figure 3.49 A).

Namely, intralaminar layer 3 and layer 5 connections occupied regions indicative of a higher $q$ than connections within layer 4, while the layer 6 connection occupied an intermediate position. However, all the subsets selected from the reciprocal layer 3 connection occupied a region indicative of a smaller $q$ than other layer 3 connections.

Consistency in parameter space was further evidenced when these independently collected data were superimposed on the plots obtained from the larger population of rat cortical connections (figure 3.56). With the exception of subsets from the reciprocal connection in layer 3, all connections fell in the regions of the plots characteristic of their laminar class. In the plot of Failure rate against M however, a shift towards larger mean amplitudes could be distinguished, that appeared consistent over subsets from all layers (figure 3.56 B).
Figure 3.55: Comparison of connections between pyramids recorded in different layers. Measurements from selected data subsets are plotted. Colour codes the layer in which the pre- and postsynaptic neurons lay (key in A). A: EPSP Coefficient of Variation plotted against Mean amplitude. B: Failure rate against Mean amplitude. C: Variance against Mean amplitude. D: Variance/Mean amplitude against Mean amplitude. Points from layer 3 (red) and layer 5 pairs (yellow) lie in a region of parameter space indicative of a larger $q$ than those from layer 4 (green). Points from layer 6 (blue) lie between. Both connections between a reciprocally connected pair of layer 3 pyramidal cells constituted an exception to the described pattern, with a combination of smaller, and less variable, EPSP amplitudes than other layer 3 connections.
Figure 3.56: Comparison of cortical functional landscapes from independently recorded connection populations. Measurements from all selected subsets of recorded EPSPs (circled dots) are plotted, superimposed on the plots obtained from a larger population (background). Colour codes the layer in which the pre- and postsynaptic neurons lay (key in A). A: Coefficient of Variation plotted against Mean amplitude. B: Failure rate against Mean amplitude. C: Variance/Mean amplitude against Mean amplitude. With the exception of one reciprocally connected pair of layer 3 pyramidal cells, the subsets from an independently recorded and analysed population did lie in the regions of parameter space corresponding to their laminar classes.
4.0 DISCUSSION

This section opens on methodological considerations regarding the experimental and computational techniques used in the course of the present studies.

Secondly, the contribution of the electrophysiological recordings performed during this PhD to the current description of basic synaptic properties within the neocortical microcircuitry is reviewed.

Thirdly, the insights provided by the discovery of a functional landscape for cortical connectivity and its description through the simple binomial model are detailed.

Finally, the conclusions drawn from the exploration of the predictable behaviour of the postulated dynamically stochastic synaptic connections are specified.

4.1 METHODOLOGICAL CONSIDERATIONS

4.1.1 Outline of the methodology

Paired intracellular recordings with biocytin filling in neocortical slices is a comprehensive technique for the examination of neuronal connectivity, the properties of synaptic connections and the unambiguous identification of the cells involved and their morphological features. The combination of electrophysiological recordings with anatomical analysis of synaptically connected pairs of neurones contributes indispensable information to the mapping of these neuronal networks that support consequential signal processing for the organism.
The recordings sought by the study described in this thesis were between excitatory pyramidal cells and between pyramidal cells and inhibitory interneurones in the layers 2/3, 4, 5 and 6 of the primary visual and somatosensory regions of the adult rat neocortex. The study attempted to correlate the morphological features of synaptically connected pairs of neurones with identified differences or similarities in their physiological properties, connection probability and synaptic dynamics.

A phenomenological model of synaptic transmission combining short term dynamics and release stochasticity was developed, and the resulting simulated electrophysiological behaviours for different sets of binomial and dynamic parameters examined in the context of experimentally inspired protocols.

A methodological framework was also designed for obtaining a functional description of the recorded cortical excitatory connections through the simple binomial model of synaptic transmission.

The suitability and limits of this simple binomial model-based methodological framework in providing a functional description of connections with heterogeneous release properties were, then, investigated in computational simulations.

The functional properties of excitatory synaptic connections in different neocortical layers were compared in adult rat and cat, and estimates of the binomial parameters best explaining these functional properties proposed.

4.1.2 Experimental techniques

The slice preparations, electrophysiological and histological techniques used, whilst arguably being the most suitable protocols for the inclusive study of intercellular connectivity, synaptic dynamics and cellular morphology, by
their very nature introduce artefacts and biases to the observations made. Notably because the neurones under examination are displaced from their natural in-vivo environment. Experimental design, particularly related to the use of acute cortical slices, therefore has several implications with respect to the data obtained.

The slicing procedure may introduce several potential biases in connectivity ratio estimation through the cutting of neuronal processes, because the sizes of some dendritic and many axonal arbours are on the order of or much larger than the typical section thickness (Stepanyants et al., 2009). Such severance limitssynaptically connected neurones to relatively closely neighbouring pairs of cells within a relatively undamaged thus healthy band of tissue within the depth of the slice. Slicing of the cortex may therefore introduce a potentially profound underestimation of the levels of connectivity, particularly over greater distances, when compared with the natural state in-vivo (Markram et al., 1997; Thomson and Lamy, 2007). The recording techniques used in this study however allowed the use of slices that were thicker (450-500 μm) than the typical (300-350 μm) section thickness used for performing in-vitro intracellular recordings. This could therefore be expected to help making a closer-to-native state of the network available to investigation.

On the other hand, deafferentation by slicing has been found to promote one form of dendritic spine plasticity as well as sprouting of axonal processes in the hippocampus when compared with perfusion fixed tissue (Kirov et al., 1999). This occurs during the first 1-2 hours after slice preparation, and the processes formed appear to support intact synapses with both pre- and post-synaptic densities. Plasticity resulting in spontaneous and activity-driven synaptic rewiring have also been demonstrated, in slices
of the young rat neocortex (Le Bé and Markram, 2006). These have obvious implications for the validity of all slice-based experimentation. The high degree of specificity observed in the properties of neocortical connections however, suggests that synapses conform to determinants largely dictated by the identity of the partner cells.

The quality of the recordings, including the cells selected, the recording duration and the stimulus protocols applied, influence which frequency-dependent synaptic properties may be studied at each connection. Even when the dynamic behaviour is dominated, for example, by depression, facilitation may still be contributing and can be revealed by this analysis.

The data from connections recorded in the course of this study, as well as a large volume of experimental data previously collected through identical protocols, were available for detailed analysis. This provided the opportunity for a better understanding and practical mastering of the methods through which the complete data analysis was performed. Ultimately these were expected to help achieve consistency in performing both recordings and analysis, thus maximising the relevance of drawn comparisons.

### 4.1.3 Computational techniques

One major methodological concern in the studies presented in this thesis was the applicability of binomial model-based analysis to the experimental data.

Throughout this work, the variability in quantal amplitude affecting our fitting methods was considered to arise solely from intersite differences (known as type II variance). However, intrasite variance (type I) may also contribute (Clements and Silver, 2000; Scheuss and Neher, 2001).
Intrasite variability may arise from fluctuations in the size of quantal events from one release event to the next, and asynchrony in their latency. The variance–mean relationship can accordingly be modified to accommodate intrasite variability that increases linearly with release probability (Saviane and Silver, 2006).

Low quantal variance was found to be an important property for successful binomial fitting, with levels above 30% precluding the applicability of fittings (Hardingham et al., 2007). At hippocampal synapses, the amplitude distribution of evoked currents coming from a single release site appears Gaussian, with mean CV values around 30% (Gulyas et al., 1993; Arancio et al., 1994). Type I quantal CV was measured to be ~40% at hippocampal single-site connections, while intersite quantal CV was ~37% at multi-site connections (Biro et al., 2005).

The distribution of quantal sizes is narrower in the adult than at immature synapses (Wall and Usowicz, 1998). At cortical connections of P19-P27 rats, the CV of quantal variance was measured to be between 0 and 41%, with an average of 17%, for those connections that could be fitted with a binomial model (Hardingham et al., 2010).

The effect of type II variance on $n$ estimation through Variance-Mean analysis has been shown through simulations to be larger than that of type I variance (Scheuss and Neher, 2001). Similarly, considering type I and/or type II quantal variance improved binomial based fittings at neocortical connections (Hardingham et al., 2010), but final estimates were not significantly different.

Therefore, although drastically different effects of type I variability on the results provided by the fitting methods used cannot be excluded, the levels of type II variability considered here (~40%, table 2.1) were expected
to put sufficient strain on the simple binomial-based fitting methods to allow their discriminatory power to be evaluated in that context.

The parameters used in Monte Carlo simulations were within the range indicated by the fitting methods when applied to experimental data from cortical connections. This allowed the discriminatory power of the fitting methods to be investigated within this range of interest. Furthermore, it is expected that larger values of release sites would improve the accuracy of the fitting methods through a relative narrowing of the postsynaptic response amplitude range, while the mean quantal size acts mainly as a scaling factor. The assumed values of release probability were designed to span two functionally relevant ranges, one for connections with low release probability (mean $p \leq 0.5$), the other for connections with high release probability (mean $p \geq 0.5$). Finally, the relative range of probabilities at any one $p$-condition was restricted such that the uniform distribution of values around the highest mean $p$ (0.8) would not exceed functionally relevant values (i.e. all sites may contribute to the variability).

More general strategies could be pursued to extend this work that would, for example, consider less constrained distributions of release probability such as $\beta$-distributions (Clements and Silver, 2000).

It was not known whether differences in $q$ and $p$ between synapses involved in the connection could confound the estimation of mean $q$ and mean $p$, from experimental data.

To determine therefore, whether more complex binomial models in which $q$ and $p$ differed between synapses would confound the analysis, data
subsets from Monte Carlo simulations of a simple and five complex models were analysed (section 3.4).

Although potent convergence was usually seen upon fitting experimental as well as Monte Carlo generated data, these methods were not designed to reject alternative quantal parameters for the individual connections on statistical grounds.

These studies demonstrated that the analysis performed could not distinguish between cases of uniform $p$ or $q$ and cases of variable $p$ or $q$ with the same mean values, and that the estimates obtained were reasonable estimates of mean $p$ and mean $q$ in all cases. In most cases therefore, simple binomial models appeared to describe well many intracortical excitatory connections.
4.2 CORTICAL MICROCIRCUITRY

The diversity and similarities in neuronal and synaptic electrophysiological properties within the neuronal circuitry of primary sensory areas in the adult neocortex were investigated using paired intracellular recordings with biocytin filling.

4.2.1 Primary sensory regions of the neocortex

The studies presented in this thesis considered data collected from both the primary visual cortex and from the primary somatosensory cortex excluding the barrel field (see section 2.3.7). Interlaminar differences in the selective connectivity and the statistics of synaptic communication were thus established across these two areas.

The arealization of neocortex into regions specialised in processing signals originating from different sensory system is phylogenetically ancient (Krubitzer et al., 2003). In comparing the known properties of primary visual and somatosensory cortices however, one realises that most of the differences that can be described derive as variations from common organising principles (Rockel et al., 1980; Linden and Schreiner, 2003; Martin and Douglas, 2004).

In other words, while the details of afferent and efferent connectivity patterns, cellular receptive fields, response properties, morphological features, etc. are found to vary between primary sensory areas of the neocortex (Dykes, 1978; Lund et al., 1993; Elston, 2002; DeFelipe et al., 2002; Sherman and Guillery, 2001; Linden and Schreiner, 2003), such properties are still inherent to both primary visual (V1) and somatosensory cortex (S1).
Hence, the continuous topographic transition between circuits processing nearby orientations in V1 appears in stark contrast with the organisation of S1 into more discrete and separated circuits corresponding to individual whiskers in the periphery (Miller et al., 2001). Both sensory systems however, establish overlays of multiple topographic projections from the entire peripheral sensory receptor sheets (Dykes 1978; Mountcastle, 1997; Linden and Schreiner, 2003). While the patterns of this patchy connectivity vary (Lund et al., 1993), spatial periodicity can also be characterised (Burkhalter, 1989; Burkhalter and Charles, 1990).

Each of these cytoarchitectonic regions thus receives external connections relaying a specific set of primary afferent fibres (Purves et al., 2004; Catania and Henry, 2006; Lubke and Feldmeyer, 2007). However, irrespective of the cytoarchitectural area and sensory modality, clusters of termination and neuronal circuits conform to a modular organisation in functional columns (Mountcastle, 1957; Dykes, 1978; Mountcastle, 1997). This results from the migration of neurones into radial columns during development (Rakic, 1988), following a similar process in both regions.

The number of neurones, pattern of synaptic organisation and design of these iteratively repeated modular microcircuits differ between areas (DeFelipe et al., 2002), but invariant principles still emerge regarding their laminar and tangential organisation. Thus, information flow is mostly relayed in a vertical direction (Mountcastle, 1997; Tarczy-Hornoch et al., 1999; Feldmeyer et al., 2002), and its intracolumnar transformation (Hirsch and Martinez, 2006) involves mostly conserved preferential intra- and inter-laminar connections between ubiquitously specific cell types (McCormick et al., 1985; Zhang and Deschenes, 1997; Callaway, 2002; Thomson and Bannister, 2003; Hooks et al., 2011).
While the number of inhibitory cells also varies between different areas, the ratio of symmetric to asymmetric synapses remains remarkably constant (Douglas and Martin, 2004), as well as the overall synapse density (Beaulieu and Colonnier, 1985; DeFelipe et al., 2002).

The conservation of some organising principles can also be exemplified by the strong parallels that exist with the hippocampus. Although the actual neuronal circuits differ from this allocortical structure in a variety of ways, similar cellular subtypes are described that connect following similar rules of selectivity (Somogyi and Klausberger, 2005).

It is, finally, unclear how inter-areal differences may compare with inter-species (Krubitzer and Kahn, 2003) or even intra-species differences (Adams and Horton, 2003; Krubitzer and Kaas, 2005).

Therefore, functionally as well as anatomically, interlaminar differences are seen to be persisting across areas, whereas interareal differences may be seen as variations around organising principles that remain to be exhaustively circumscribed. In this respect, it cannot be excluded that the examination of a larger number of connections from each area could reveal numerical differences between these two neuronal populations within the homogeneity described here.

4.2.2 Firing patterns

The frequency filtering capabilities of synaptic short term dynamics and their association with the firing properties of the presynaptic cell, contribute to the information transferred selectively at each stage across cortical circuits (Thomson and West, 1993; de la Rocha et al., 2002; Williams and Atkinson, 2007).
Several specific firing patterns were identified from neurones located in each of the cortical layers where recordings were performed. These correlated with the morphological features and laminar location of the cells revealed after histological processing. Subtle differences in the action potential waveform and firing pattern upon different levels of membrane depolarisation provided reliable clues about the cell type that was being recorded.

The firing patterns encountered and the laminar distribution of the identified cells did not differ substantially from those commonly reported in the literature (McCormick et al., 1985; Nowak et al., 2003; Markram et al., 2004).

A spectrum of firing patterns in cortical excitatory cells ranged from little spike frequency adaptation such as layer 6 corticothalamic cells, to stereotypical burst firing most commonly in layer 5 cells but also apparent in some layer 3 cells. Between these two extremes were more diffuse types, with a range of so-called "burst adapting" patterns more abundant in more superficial layers with distinct laminar flavours regarding their action potential and initial burst waveforms.

Inhibitory interneurones were usually distinguished from pyramidal cells on the basis of a shorter capacitance time constant, higher firing frequency capability with an interrupting or non-adapting pattern and a deep fast hyperpolarisation following each spike.

Consistency with previously reported firing characteristics in all layers recorded (McCormick et al., 1985; Thomson et al., 2002; Beierlein and Connors, 2002; Llano and Sherman, 2009) indicate that our recording conditions were suitable for our endeavour of exploring physiological properties at the scale of the cortical network.
4.2.3 The probability of synaptic connections

Paired intracellular recordings reveal the selectivity that appears to govern the formation of cortical synapses. Resulting insights may usefully be compared with what could be expected in anatomically accurate random connectivity models (Kalisman et al., 2003; Binzegger et al., 2004; Stepanyants et al., 2007).

The frequency of synaptic interactions between specific cell types within and across cortical layers was assessed via the number of tests between pairs of recorded neurones required before a synaptic connection was observed. This provided a "hit rate" expressed as a ratio (figure 3.2).

The proportions of connections found between cells in the same layer that displayed an adapting firing pattern were consistent with previously published assessments in the layers 2/3 (Mason et al., 1991; Thomson, 1997; Yoshimura et al., 2005; Kapfer et al., 2007; Ren et al., 2007), 4 (Petersen and Sakmann, 2000; Beierlein et al., 2003; Maffei et al., 2004; Bannister and Thomson, 2007), and 5 (Markram et al., 1997; Thomson et al., 2002).

A sufficiently large population of pyramid-pyramid connections was tested in layer 2/3 for an estimate of the prevalence of reciprocal connections to be proposed. This provided a ratio of 3:26, very similar to 7:61 found in Holmgrem et al. (2003). For comparison, a larger prevalence of reciprocal connections between spiny cells was reported in other layers, with ratios of 8:30 reciprocal connections within layer 4 (Tarczy-Hornoch et al., 1999) and 42:138 within layer 5 (Markram et al., 1997).

The sparse intralaminar connectivity evidenced between pyramidal cells and interneurones in the recordings performed for this study, owes to the search strategy that was applied, as interneurones were not more particularly
sought. High prevalence of connections onto interneurones was nevertheless apparent in the case of layer 2/3 (6:16), and within layer 6, from corticothalamic cells (non-adapting in figure 3.2), as had been described previously (White and Keller, 1987; Staiger et al., 1996; Tarczy-Hornoch et al., 1999; Beierlein et al., 2003; West et al., 2006).

One translaminar connection identified here, involved cells with an adapting firing pattern in layer 3 forming excitatory connections onto non-adapting, i.e. burst firing, layer 5 cells, described as a highly focussed and extremely dense, descending excitation pathway (Thomson and Bannister, 1998; Thomson and Lamy, 2007) within the cortical column.

4.2.4 Postsynaptic potential properties

While recordings were performed over similar ranges of postsynaptic membrane potentials (figure 3.3 A), some differences existed in the properties of the average single EPSP from connections between cells in the different layers (table 3.1).

Peak EPSP amplitude appeared larger on average at connections onto cells in layer 5, and tended to be smaller on average at connections onto adapting cells in layer 3 or at layer 6 connections (figure 3.3 B). These observations were consistent with previous results (Brémaud et al., 2007).

A novel observation in this study was the difference in mean EPSP amplitude found between layer 2/3 connections onto adapting cells and layer 5 connections, possibly because it had not been considered with that level of segregation (but see Reyes and Sakmann, 1999; Hardingham et al., 2010).

The width at half amplitude also appeared as a distinctive feature between laminar connection classes regarding single EPSP properties, even providing ground for intralaminar distinctions.
Connections in layer 6 had width at half amplitude that was indicated to be shorter than layer 3 connections onto adapting cells and layer 5 connections. Such a trend can be recognised in earlier studies. Most of the previously reported layer 6 connections between excitatory cells have width at half amplitude shorter than about 13 ms which, on the other hand, appears to be a lower limit for this type of connections in the other layers (Thomson and Lamy, 2007 for a review).

Width at half amplitude was found to be about 3 fold shorter at connections onto interneurones (5.6 ± 4.3 ms, n=4) than at connections onto adapting cells (16.6 ± 5.0 ms, n=6; P<0.01, Student’s unpaired t-test; figure 3.3 C), in layer 3.

Within our sample, the 10-90% EPSP rise time was not found to be a distinctive feature for excitatory connection classes in the different cortical layers (figure 3.3 D), in contrast to previously published studies (Buhl et al., 1997; Reyes et al., 1998; Thomson et al., 2002).

4.2.5 Short term synaptic dynamics

The recording method, age of preparation, and temperature influence the strategies commonly employed to assess the dynamic properties of connections and, therefore, the data obtained. Most paired whole-cell recordings take advantage of the facility with which presynaptic action potentials can be reliably activated by brief, large amplitude current pulses, at an interval or frequency determined by the experimenter, without compromising the postsynaptic recording (e.g, Feldmeyer et al., 2006). Typically, therefore, these studies select one or a few presynaptic frequencies. These frequencies are often relatively low to allow the slower postsynaptic responses typical of young tissue (Ali et al., 2007) to subside.
before another is elicited. While this allows large data sets with consistent interspike intervals it precludes more detailed analysis of the dynamic profile.

Paired sharp electrode recordings suffer from capacitance coupling which generates artefacts in the postsynaptic recording when the flow of current in the presynaptic electrode changes. The application of brief current pulses of sufficient size to elicit action potentials is, therefore, not an option. Instead, longer current pulses with a range of shapes and amplitudes can be injected to generate a range of presynaptic firing frequencies and patterns. Specifically designed post hoc analysis is then required to identify relevant and distinctive features in the postsynaptic responses from the broad spectrum of synaptic regimes uncovered across non-discreet ranges of interspike intervals and less constrained firing patterns.

When both neurones are stable and recordings can be maintained for long periods (1–4 hours), large datasets that include synaptic potentials elicited with a wide range of presynaptic firing frequencies and patterns are generated. These recordings allow the fine details of the recovery from synaptic facilitation or depression to be analysed and have revealed great complexity in the time course of these processes (Thomson and West, 2003; West et al., 2006; Ali et al., 2007; Bannister and Thomson, 2007). In particular these protocols allow the study of very short presynaptic interspike intervals in adult tissue (<10 ms), a frequency range that is not commonly addressed in paired whole-cell experiments, but one that is, nevertheless relevant to sensory processing in vivo (König et al., 1996; Nowak et al., 1997; Cardin et al., 2010). The injection of ramps of current, for example, revealed useful in eliciting a range of presynaptic firing patterns, particularly in strongly adapting cells.
In this study, the characterisation of short term dynamic properties at the recorded connections was not the only parameter set accessible from the data. The fluctuations in EPSP amplitude of successive responses to trains of presynaptic action potentials superimposed on such dynamic profiles, were captured in subsets of data consequently associated with a range of release conditions, used to estimate binomial characteristics.
4.3 BINOMIAL PARAMETERS FOR CORTICAL CONNECTION CLASSES

Classically, quantal and fluctuation analysis require experimental protocols that generate large data sets of responses to single action potentials over fixed, stable, recording conditions (Redman, 1990; Clements, 2003). While ensuring for stability in release conditions is essential in all analysis, the one used in this study does also allow, and utilises, the study of synaptic dynamics. The collected data can thus be relevant beyond the specific purpose of fluctuation analysis. Although stability and consistency criteria lead to the exclusion of many datasets, some of those recorded during this study were thereby indicated as suitable for fluctuation analysis (section 3.5).

Subsets selected for parameter estimation thus included EPSPs recorded within a narrow range of resting membrane potential (e.g. figures 3.32-3.34). The standard deviation time course provided both an indication about functional homogeneity of synaptic transmission at any one connection, and of the portion of the EPSPs at which fluctuations were the largest. Connections at which CV^2 analysis indicated a postsynaptic origin for differences between the selected subsets of EPSPs were also excluded from further analysis.

Binomial model-based methods have been less widely used for estimating n, p, and q than to assess the site responsible for changes in mean response amplitude. Only one previous study in adult cat neocortex had previously compared neocortical connections this way (Tarczy-Hornoch et al., 1999). Larger numbers of connections and data subsets and a wider range of connection classes were available here.
One major methodological concern in the studies presented in this thesis was the applicability of binomial model-based analysis to the experimental data. Strong correlations for fits of data from single connections with the four statistical relationships and correlations between parameter estimates obtained with these four methods (figure 3.52) indicated that binomial models described the type of data analysed well. The parameter estimates and ranges summarised here (table 3.2) could therefore be useful in models of cortical circuitry.

4.3.1 Functional landscape of cortical connectivity

The principle outcome of this study is the differences in the regions of parameter space occupied by different classes of cortical connections (Brémaud et al., 2007), whether the coefficient of variation, the failure rate or the ratio of variance to the mean amplitude is plotted against the mean EPSP amplitude (section 3.5; figure 3.49), or the estimated mean quantal amplitude is plotted against the estimated number of release sites (figure 3.54).

More sophisticated methods will be required to distinguish whether these differences result from the number and type of receptor, synapse location and/or dendritic properties and filtering. However, such differences in the functional profiles of laminar classes of excitatory connections have been identified in subsequent independent studies in the young adult rat neocortex (Hardingham et al., 2010), and the same kind of differences were found within the hippocampal circuitry (Mercer et al., 2010).

In rat, intralaminar layer 4 pyramid–pyramid connections stood out (table 3.2), exhibiting a lower mean quantal amplitude ($q$) than connections in other layers despite a similarly wide range regarding the estimated numbers of release sites ($n$).
Layer 6 corticothalamic pyramid outputs also stood out. Although estimated mean quantal size was similar to that in other layers, both their estimated number of release sites and mean probability of release were particularly low for pyramid-pyramid connections, the low $p$ correlating with these being the only pyramid–pyramid connections consistently described as facilitating in neocortex (Beierlein and Connors, 2002; Beierlein et al., 2003; Mercer et al., 2005).

The independently collected data from pyramid-pyramid connections recorded in the course of this PhD, similarly occupied the previously identified regions of the parameter space for the different connection classes (figure 3.56). One exception appeared however, regarding the data from both connections between two reciprocally connected layer 3 pyramidal cells. These displayed combinations of low variability in EPSP amplitude as well as failure rate and low $M$, that located these values within the region of parameter space previously identified as occupied exclusively by layer 4 connections. A lower mean amplitude of the postsynaptic response has been previously reported at reciprocal connections between layer 2/3 pyramidal cells (Mason et al., 1991). It is possible therefore that reciprocal connections would constitute intralaminar classes with specific functional profiles.

The larger values of mean quantal amplitude at pyramid–interneurone compared with pyramid–pyramid connections explain the larger coefficients of variation of pyramid–interneurone EPSPs that have been described previously, but not explicitly explored (Thomson et al., 1995; Deuchars and Thomson, 1995; Beierlein et al., 2003; Reyes et al., 1998). This large $q$ may result from the shorter electrotonic lengths of interneuronal dendrites (Thurbon et al., 1994; Chitwood et al., 1999; Nörenberg et al., 2010) and/or different numbers and types of receptors.
The differences found in the regions of the parameter space occupied by different cortical connection classes suggests that different, layer specific, combinations of \( n \), mean \( p \), and mean \( q \) are employed to achieve the range of synaptic strengths displayed.

### 4.3.2 Quantal amplitude

In this study, the measure of quantal amplitude was derived from the magnitude of the postsynaptic responses as recorded at the cell body. It therefore reflects the average result from the interaction between determinants of the postsynaptic depolarisation upon release (see section 1.1.4), across all the synaptic contacts constituting a connection.

Monte Carlo simulations indicated that non-uniformity in \( q \) across release sites could be expected to be a major component in loss of accuracy for the estimation of binomial parameters conducted (section 3.4). This could result from release sites having different characteristics, or from identical sites located at different electrotonic lengths along the dendritic tree, from which signals would be differentially filtered (see section 1.1.4). The similarity in postsynaptic response waveforms however was indicated by considering the standard deviation time course of the EPSP (e.g. figures 3.32, 3.41, 3.45). Since the shape of the standard deviation time course matched that of the average EPSP, an equivalent electrotonic distance for all inputs can be assumed (Redman and Walmsley, 1983b; Thomson et al., 1993). This is expected from an histological and ultrastructural perspective, as synaptic contacts establish over restricted portions of the dendritic tree, innervating specific subcellular compartments of their connection partner (Di Cristo et al., 2004; Thomson and Lamy, 2007).
With the three methods based on measures of variability in response magnitude (Coefficient of Variation, Variance, Variance/Mean), estimates of q demonstrated lower coefficients of variation than estimates of n, suggesting that q is more accurately estimated than n by these methods. This may reflect the fact that, functionally, this parameter only affects the mean amplitude in the binomial relationships:

\[ M = n.p.q \quad \text{whereas} \quad CV = \sqrt[2]{\frac{1-p}{n.p}} \quad (\text{see section 2.4.2}) \]

The accuracy of experimental measures therefore intervenes on a single dimension of the plane explored by the fitting procedures, whereas both n and p estimates would affect measures of both variability and mean amplitude. More precise results may also simply reflect a tighter distribution of quantal size owing to structural uniformity at individual connections (Zhang et al., 2009; but see Koester and Johnston, 2005).

Contrary to some methods based on histogram fitting, our estimates of quantal parameters do not involve estimates of q from measurements of spontaneous and miniature synaptic events. This implies that the individual estimates obtained do not attempt to describe a population of inputs whose heterogeneity might owe to the diversity in presynaptic partners.

Our estimates of quantal size (table 3.2) are consistent with previous reports indicating a range of values between 0.1 and 0.8 mV (Markram et al., 1997; Buhl et al., 1997; Feldmeyer et al., 2006; Hardingham et al., 2010).

Results obtained from similarly inspired methods suggested that, in the young rat neocortex, transmission at the stronger synaptic connections is mediated by multiquantal release from their synaptic contacts (Loebel et al., 2009). This prompted these authors to reconsider the interpretation of the parameter n as the number of readily releasable vesicles rather than the
synaptic contacts or active zones, whereas the single vesicle hypothesis (section 1.2.2.1) received support at an hippocampal synapse (Biró et al., 2005).

An array of factors may preside over the determination of the quantal amplitude, that include the cleft concentration in transmitter, the receptor number, types and kinetics, the postsynaptic driving force, as well as passive and potentially active dendritic filtering. Relative invariance of $q$ estimates compared to $n$ estimates within a class of connections, might therefore indicate that tighter biological constraints apply to these components in relation to the identity of both synaptic partner cells.

4.3.3 Number of release sites

The interpretation of the parameter $n$ in quantal analysis is less straightforward than quantal amplitude since it describes the average number of quanta available for release upon recruitment by a nerve impulse. Under the assumption that the transmitter content of a single vesicle might be released at each release site, $n$ should correlate well with the number of synaptic contacts between two cells. Recent evidence from fluctuation analysis conducted in the young rat neocortex indicated values of $n$ much larger than the reported number of synaptic contacts at these connections, suggesting that multiquantal release might occur (Loebel et al., 2009). The estimates of $n$ yielded by our analysis in adult rat neocortex however were well below such values for all the connection classes described (table 3.2).

The issue of the anatomical correlates of $n$ also arises because $n$ and $p$ are not entirely independent parameters. Provided that the probability of release would be either null or very close to zero at one or several of the release sites, finite samples of postsynaptic responses would, for instance,
necessarily either not comprise the contribution of these units to amplitude fluctuations (Redman, 1990), or overestimate it when present by chance. Statistical analysis is therefore not designed to detect nor consider such "silent" (Liao et al., 1995; Rumpel et al., 2004; Ashby and Isaac, 2011) or "whispering" (Lisman et al., 2007) synapses. The consequences of synaptogenesis (Petrak et al., 2005) and synaptic rewiring (Le Bé and Markram, 2006) in acute slices are not well understood and it is possible that such phenomena generate and involve these forms of contacts essentially invisible to statistical analysis. Our account of $n$ is thus very much functional, i.e. sufficient to account for the characteristics considered, rather than aimed at an accurate anatomical prediction.

Additionally, both $n$ and $p$ stand as candidate causes when a presynaptic locus for changes in synaptic efficacy is predicted through the $CV^2$ analysis. One of the assumptions for applying the equations used in fitting our data is that changes in $p$ alone underlie the different release conditions portrayed in the selected subsets of amplitudes. Lower release probability conditions might withdraw some release sites from detection by the analysis, resulting in a change in the estimated value of $n$. However, when the release probability was lowered experimentally by applying $\omega$-conotoxin (figure 3.48), the estimates of $n$ and $q$ obtained were consistent with those obtained in the control conditions.

Monte Carlo simulations indicated that large differences in these functional estimates would be robust beyond some of the simplifications inherent in a strictly binomial model of release. This was particularly the case for non-uniformity in release probability (figure 3.28). The simulations also confirmed that methods based on measures of the variability in response magnitude gave more reliable estimates of $n$ and $q$ over the range of higher
release probabilities, whereas the Failure rate against M method was more reliable over the range of lower release probabilities (figure 3.31). Indeed, plots of expected relations (figure 2.4) indicate that the separation between model curves of variability measures is minimal for large differences in $n$, unless $p$ is high.

When the quantal parameter estimates obtained across the larger population of connections using the different fitting equations were compared (figure 3.52), the method relying on the failure rate appeared to provide consistently lower estimates for the number of release sites.

The number of release sites can be underestimated by variance-based methods when quantal variance is present (section 3.4.3.1; Scheuss and Neher, 2001). Thus, the bias in estimated values of $n$ from the failure-based method might not arise from quantal variance.

When the noise variance was subtracted from the measures of variability for a subset of connections, the estimates of $n$ from the 3 other methods were not found to be consistently lower (section 5.3).

One simple explanation would be that this method, by relying on the measure of failures of release, may only be accurate at connections with a lower number of release sites (see section 3.4.3.3 and figure 3.31). It is possible therefore, that the fewer estimates of $n$ that could be obtained using this method would be biased towards lower values.

The functional estimates obtained here in adult tissue (table 3.2), indicated that amplitude fluctuations at excitatory connections were best described by values of $n$ that were larger on average than the average number of contacts identified in histological studies, and larger than estimates obtained in the young rat, for the same laminar classes (Deuchars et al., 1994; Markram et al., 1997; Feldmeyer et al., 1999, 2006; Cowan and
Stricker, 2004; Saez and Friedlander, 2009; Hardingham et al., 2010). The values of our estimates appear closer to those obtained in studies that were concerned with adult tissue (Mason et al., 1991; Tarczy-Hornoch et al., 1999).

These large $n$ values come in contrast with Monte Carlo simulation results, which indicated that, when applied to more complex binomial models of release, the analysis would provide underestimates of the number of release sites rather than overestimates (figure 3.28). Therefore the potential bias introduced by using a simple binomial-based analysis does not appear to explain that functional estimates be larger than the number of histological contacts.

Taken together with consistency in quantal amplitude estimates (section 4.3.2), this would suggest that the amplitude fluctuations at most neocortical excitatory connection classes could be generated at synaptic contacts containing more than one release site. However, the identification and confirmation of all the synaptic contacts established between two cells is a demanding process, the full completion of which can rarely be ascertained. Microscopical examinations might therefore, on the whole, be expected to provide an underestimation in that respect.

Finally, the larger variability in $n$ estimates found within the cortical connection classes might be an indication that the associated biological processes are less tightly constrained by intercellular recognition than can be the case for $q$ and $p$. 
4.3.4 Release probability

Seminal work from Bernard Katz and colleagues in the 1950s established that most of the variability in synaptic transmission reflects the stochastic nature of the exocytotic mechanisms (Katz, 1969). It follows that transmitter release at an individual release site is often adequately described as a probabilistic all-or-none event, a “success” then resulting in a quantal (unitary) postsynaptic response. The release probability therefore aggregates a number of dynamically decisive presynaptic factors, from the reliability of action potential conduction, to the types of calcium channels and their interaction to a fully assembled release machinery, as well as phospholipidic membrane compositions, that actuate vesicular competence, across all synaptic sites established by one cell onto another.

Binomial models used as mathematical formalisations of synaptic transmission require further assumptions about the probability of release at individual release sites. In a simple model, release probability is postulated to be identical at all the release sites constituting a connection, and the stochastic release events are assumed to occur independently of one another upon presynaptic triggering.

The analysis conducted in this study required that the release probability be the parameter underlying short term synaptic dynamics, as had been suggested to be the case (Thomson and West, 1993; Thomson et al., 1993; Dobrunz and Stevens, 1997; Tsodyks and Markram, 1997; Tarczy-Hornoch et al., 1999; Oertner et al., 2002). Experimental conditions, such as the concentration of extracellular Ca\textsuperscript{2+} do affect release probability and dynamic properties. However, from the concentrations typically used in slice experiments (2.0–2.5 mM) to the lower concentrations thought to be more physiologically accurate (e.g. 1.0 mM free Ca\textsuperscript{2+}), only relatively small
differences in dynamic properties are apparent (e.g., Thomson, 1997; Thomson et al., 1993, 1995). Here, pharmacologically impairing the triggering of release confirmed that different release probability conditions spanned the regions of parameter space occupied by data from a single connection (figure 3.48). Indications from CV^2 analysis of a presynaptic origin for changes in synaptic efficacy, and the adequacy of the fits performed on data from each connection, suggested that changes in the release probability were the major contributor to dynamics at many cortical excitatory connections.

Connections for which this relation was not verified were not included. The profiles obtained in many such cases resembled those obtained in situations where changes in q were induced (e.g. figure 3.34), but with parallel curves accommodating subsets from the successive EPSPs in a train. An economical explanation is non-independent release from groups of terminals served by single axonal branches that fail on occasions to transmit an action potential. The analysis recognises the activation of all terminals served by a branch as a unitary or quantal event whose amplitude increases as p increases (see also Korn and Faber, 1991), resulting in the appearance of a smaller n and a larger, but not invariant, q.

Branch point failure, whereby the propagation of action potentials along the axon and its collaterals would be intermittent and/or partial, could confound the analysis. Propagation failures have been demonstrated in some mammalian systems (Debanne et al., 1997; Segev and Schneidman, 1999; Soleng et al., 2003). Calcium imaging studies however, conclude that they are rare in pyramidal cells of the neocortex and hippocampus (Mackenzie and Murphy, 1998; Cox et al., 2000; Koester and Sakmann, 2000). Under higher and sustained firing frequencies, propagation failure
detection in pyramidal axons was possible after ~60 spikes at frequencies between 5 and 50 Hz during plateau potentials (Meeks et al., 2005), but not via intracellular recordings of the main axon (Shu et al., 2007). It would appear therefore that robustness in action potential initiation (Chen et al., 2010) and propagation along cortical axons precludes a major contribution by branch point failures to fluctuations at these connections.

Heterogeneity in release probability has been reported for synapses in different nervous structures (Walmsley et al., 1988; Rosenmund et al., 1993; Allen and Stevens, 1994; Yuste et al., 1999). Direct imaging concluded that release probability of different axonal boutons from a pyramid contacting the same target cell varied little in the neocortex (Koester and Johnston, 2005).

Across the larger population of connections studied, a negative correlation was found between paired-pulse ratio and probability of release (figure 3.53), indicating that connections with a low release probability were prone to facilitation whereas connections with a high release probability tended to display depression. This result is consistent with many other studies (e.g. Dobrunz and Stevens, 1997; Atzori et al., 2001; Hardingham et al., 2007; Saez and Friedlander, 2009; see Zucker, 1989 for review). An additional correlation has also been demonstrated between $p$ and the size of the recycling pool of vesicles in cultured hippocampal cells (Murthy et al., 1997) or the number of vesicles docked at the active zone (Branco et al., 2009).

It has been reported that connections between neocortical pyramidal cells may work at higher regimes of release probability than most of those found in a closely related brain structure, the hippocampus (Hessler et al., 1993; Allen and Stevens, 1994; Huang and Stevens, 1997; Yuste et al., 1999; but see Mercer et al., 2010), with values pertaining to the upper third of
the probability range (Buhl et al., 1997; Tarczy-Hornoch et al., 1999). However, the potential importance of this factor for information processing across neuronal circuits (Tsodyks and Markram, 1997; Maass and Zador, 1999) and the acknowledged diversity of connection classes with specific properties in the circuits of both structures, advocated a finer grained arrangement across connection classes (Rosenmund et al., 1993; Allen and Stevens, 1994). Indeed, the present study indicates that $p$ at rest appears consistent within a type of connection, while it may differ across types (table 3.2).

Recent assessments of release probability at cortical connections between pyramidal cells provided values in the upper two thirds of the range, ranging between 0.48 and 0.88 with a mean of 0.64 ± 0.18 (Hardingham and Larkman, 1998), or 0.46 ± 0.26 (Koester and Johnston, 2005), ranging from 0.12 to 0.91 with a mean of 0.47 ± 0.20 (Hardingham et al., 2010) in young rat layer 2/3, with a mean of 0.41 ± 0.13 in layer 4 of the guinea pig (Saez and Friedlander, 2009), and 0.46 ± 0.21 at layer 5 connections in the young rat (Hardingham et al., 2010). In the latter study, an average release probability of 0.57 ± 0.22 was also estimated for translaminar connections between L2/3 pyramidal cells and layer 5 cells. Our estimates of release probability for pyramid-pyramid connections, obtained in adult tissue, spanned a range of similarly high values (0.53 - 0.86), with the notable exception of those established by layer 6 cortico-thalamic (CT) cells (0.28).

The distinctively lower estimates of $p$ reported here for layer 6 connections originating from layer 6 CT cells in the rat however, are consistent with estimates reported for layer 6 pyramidal cell synapses in layer 4, as moderately high (0.37-0.56) and very high at layer 4 cell synapses.
(0.69-0.98; Tarczy-Hornoch et al., 1999), since this cell type establishes the main axonal projections from layer 6 to layer 4 (Thomson, 2010).

### 4.3.5 Cat connections

Differences in the functional profiles of laminar connection classes were also found in the results from paired recording experiments performed in the adult cat neocortex. This is in agreement with early indications from a study where two such classes had been compared (Tarczy-Hornoch et al., 1999).

In cat, intralaminar layer 4 pyramid–pyramid connections appeared to exhibit a larger mean quantal amplitude and a narrow range of smaller values of the number of release sites over the relatively smaller number of data sets available. With the exception of layer 4, cat connections appeared to involve a larger number of smaller quanta than rat connections, possibly reflecting the larger volume available for making connections in cat neocortex.

However, the small quantal size estimated for cat layer 4 to layer 3 pyramid connections is consistent with the range of smaller values (0.04-0.4 mV in Feldmeyer et al., 2002) required to describe these connections compared with layer 4 intralaminar excitatory connections (0.15 - 0.8 mV in Feldmeyer et al., 1999) in the young rat, although layer 3 pyramid-pyramid connections had been expected to use a larger quantal size (Feldmeyer et al., 2006) which was indicated not to be the case here, in adult cat.

### 4.3.6 Connections onto interneurones

Histological studies reported, in adult rat neocortex, between 3 synaptic contacts and 12 putative contacts at deep pyramid to burst firing interneurones (Deuchars and Thomson, 1995), and in cat neocortex between
1 and 2 synaptic junctions from pyramidal cells to basket cells, 5 and 7 synaptic junctions for connections onto other inhibitory interneurone types (Buhl et al., 1997).

The earlier range does accommodate the values of our functional estimates, however the limited but rigorously examined sample presented in Buhl et al. (1997) rather challenges our values as the comparison between the estimated number of release sites at pyramid to multipolar interneurone and pyramid to bitufted interneurone connections yields the opposite result in the cat, with the former type predicted to be using 7.6 ± 5.8 (n=6) sites and the latter 2.7 ± 1.0 sites (n=3; P<0.05, Student's unpaired t-test). Were the discrepancy apparent in these small samples confirmed, it would suggest that a very different organisation prevails at these two types of connections in the cat, with pyramid to multipolar interneurone connections using a larger number of release sites per contact, while pyramid to bitufted interneurone connections could comprise a larger proportion of "silent" contacts. This would, in particular, be consistent with our estimates of their respective release probability.

Our estimates of release probability at excitatory connections onto interneurones were consistent with previous reports, with high values at pyramid to multipolar interneurones connections (Gulyas et al., 1993). Our results also closely paralleled the differences in release probability measured optically between these connections (0.64 ± 0.16, ours: 0.63 ± 0.25 in the rat, 0.49 ± 0.27 in the cat) and those onto bitufted interneurones (0.13 ± 0.08, ours: 0.24 ± 0.17 in the rat, 0.28 ± 0.17 in the cat) in the young rat neocortex (Koester and Johnston, 2005), suggesting that the functional profile of these two connection classes might contribute specific roles of the target cells for signal processing within the cortical circuit (Thomson and Jovanovic, 2010).
4.4 BEHAVIOUR OF DYNAMIC STOCHASTIC SYNAPSES

Two major properties of synaptic transmission have been evaluated experimentally in the adult neocortex throughout this study. Short term dynamics of the postsynaptic responses to trains of presynaptic action potentials were examined across ranges of interspike intervals, and the probabilistic nature of fluctuations in the amplitude of these same responses investigated. The identification of subsets of these fluctuating responses with consistent characteristics within the time course of particular synaptic dynamics, was used to establish functional profiles for individual connections, that appeared to differ between cortical connection classes.

To understand the consequences of synaptic dynamics generated through modifications in release probability better, a dynamic stochastic model of synaptic release was designed and simulations of different binomial parameter combinations performed and analysed according to protocols that replicated those used for experimental data.

4.4.1 Properties and purpose of the model

Most models for synaptic transmission adopt either a phenomenological approach addressing and matching the average behaviour experimentally described through a deterministic description (Varela et al., 1997; Markram et al., 1997; Weis et al., 1999; Dittman et al., 2000; Furhmann et al., 2004; Xu and Wu, 2005; Sun et al., 2007; Hennig et al., 2008), or incorporate one or several stochastic components seeking to provide Monte Carlo simulations of biophysically more realistic and accurate synaptic models for deciphering the succession of causal processes involved (Matveev and Wang, 2000; Franks et al., 2003; Raghavachari and Lisman, 2004; Nadkarni et al., 2010).
Both theoretical (Maass and Zador, 1999) and computational (Cai et al., 2007; Mayr et al., 2009) studies reveal the emergence of properties inherent to the stochastic character of synaptic release, properties that cannot be obtained in the equivalent deterministic descriptions of short term dynamics. According to these studies, the interplay of stochastic transmission and synaptic dynamics is particularly relevant when spike timing-based information processing is envisaged to occur via synapses.

The stochastic dynamic model was developed using an environment well distributed and recognised within the computational neuroscience community (Brette et al., 2007), the NEURON simulation environment (Hines and Carnevale, 1997). This will allow the model to be incorporated as a mechanism within detailed models of cortical networks or established deterministic models of particular connections.

The model is derived from the phenomenological model of short term synaptic dynamics from Tsodyks and Markram (1997) and Tsodyks et al. (2000), by equating “synaptic resource” to probability of release for a number of independent mechanisms equal to the specified number of release sites, and adjusting the unitary postsynaptic depolarisation to the specified quantal amplitude. Accordingly, in the model, depression following a presynaptic action potential is proportional to, and affects, the current value of release probability, which thereafter follows exponential dynamics back to its value at rest during recovery from depression. Quantal transmission itself however, happens stochastically at the presynaptic action potential arrival, according to the current value of release probability.

Our approach in simulating release sites with a dynamically regulated stochastic and quantal release, therefore explores the behaviour of
postsynaptic responses from the idealised connections postulated by the analysis that was used on experimental data. This also allows some assumptions made in performing the analysis on experimental data to be challenged, in light of results provided by the idealised connections under similar protocols.

4.4.2 Consequences of dynamically stochastic release

The principle outcome from the simulations of the stochastic dynamic model, is that peaks and troughs were present when the moving averages of the simulated single sweep amplitudes were plotted against the interspike interval (figures 3.14, 3.15, 3.16). In contrast, smooth exponential recoveries were obtained from the deterministic model (figure 3.13). This establishes simple dynamics regulating stochastic release as a potential cause for the emergence of the peaks and troughs commonly seen in experimental data (e.g. figure 3.45; also see Thomson and West, 2003; West et al., 2006; Bannister and Thomson, 2007). The quantification of relative deviation from the predicted exponential recovery from depression at those idealised connections, confirmed that larger peaks and troughs would be indicative of connections with a smaller number of release sites and a larger quantal amplitude (section 3.3.3).

Figure 3.13 also provides another interesting insight about the deterministic model. While plots of simulated EPSP amplitudes against preceding interspike intervals provided smooth distributions of values along the exponential recovery, against two preceding EPSP amplitudes, it displayed a patchy and seemingly random pattern of low and high amplitude values. This indicates that, in the deterministic model of synaptic release considered, the amplitudes of EPSPs are entirely determined by the intervals
between the preceding presynaptic action potentials. This means that individual EPSP amplitudes are then a direct reading of the immediate presynaptic history of action potential emission, they would not carry other information about synaptic history.

When data from the dynamic stochastic model were plotted in the same way (figure 3.17), most of the smooth exponential layouts was obscured. Although average amplitude increased with increasing intervals, this was not always apparent (figure 3.14). It was replaced by bands of amplitude similarity at particular time intervals, more similar in that respect to experimental results (e.g. figure 3.8). The complete dominance of the immediately preceding interval in determining an individual EPSP amplitude was also alleviated (figure 3.17 A2 and B2).

From these simple considerations, it appears that dynamically regulated stochastic release prevents a transmission that would be fully informative only about the recent history of presynaptic spike timing seen with deterministic dynamics. Therefore, despite the unpredictable nature this property can confer on a model of synaptic transmission (Zador, 1998), it also makes room for more than one type of information to be conveyed (Pfister et al., 2010) through the amplitude of postsynaptic responses. In particular it is possible, although we did not investigate this matter, that more information can be conveyed about preceding synaptic responses, since in the dynamic stochastic model these cease to be a strict product of the preceding interspike intervals.
4.4.3 Subset selection

Moving distributions of amplitudes plotted against the preceding interspike interval for simulations of connections with dynamic stochastic synapses allowed the identification of the data subsets for which the distribution was closest to the one predicted by the binomial model (figure 3.19 and 3.20). This indicated that the selection of representative subsets is a necessary step when performing the analysis, as all possible subsets may not conform to binomial predictions despite resulting from simulations of such idealised connections.

That subsets of amplitudes with optimal statistics are located at transiently stationary portions of the moving average against interspike interval plots, was indicated by the fact that the distance to target pairs of statistics (CV and M expected from the simple binomial at a particular release probability) was minimal for the larger subsets with the lower moving first derivative of amplitude (figure 3.21 and 3.22). This implies that the method of selecting subsets within the peaks and troughs of the moving average against interspike interval, that was used in the analysis of experimental data, is consistent with, and efficient for, our hypothesized model of synaptic connections.

4.4.4 Power spectra of moving average trajectories

Whether the peaks and troughs in these moving averages were generated in oscillating patterns according to certain preferred frequencies was examined using the power spectra from bootstrap sampling of simulated data (section 3.3.6). None of the model specifications considered appeared to display a consistent pattern of peaks at specific frequencies in the power
distribution over resampling (figure 3.23). This indicates that a stochastic-dynamic mode of release does not induce the peaks and troughs to be organised into oscillations at specific frequencies.

Instances of the stochastic dynamic model however, appeared to display moving average plots with more power over a range of frequencies between 1 and 100 Hz, that did not appear in data from simulations of the deterministic model. More power appeared to be displayed over this frequency range, in the data from simulations of the model instances with fewer release sites and a larger quantal amplitude, than in simulations of the model instances with more release sites and a smaller amplitude. This is again an indication that the generation of peaks and troughs resulted from the stochastic nature of release, and that connections with fewer release sites and a larger quantal amplitude display such interruptions in the moving average plots to a greater degree.

Overall, throughout this study of the behaviour of models with dynamically stochastic release, no obvious differences were identified between simulations in which the dynamics at individual sites were release dependent or release independent.
4.6 FUTURE STUDIES

Paired intracellular recordings with biocytin filling using sharp microelectrodes is a uniquely tailored method for the comprehensive exploration and description of cortical microcircuits in adult tissue, and computational techniques currently our most powerful tool in the reverse engineering of their many capabilities. The combined approach of maintaining neuronal and silicon experiments in a conversing stance that was deployed across the studies included in this thesis, allows a constant bilateral refinement in the pursuit of answers to neuroscientific questions. Though undoubtedly demanding separately, these techniques could effectively be applied in combination further, to address questions that this study did not encompass.

The dynamic-stochastic model developed can thus be used to investigate the consequences of non-independency in release at multiple sites, the interplay of simultaneous and realistic depression and facilitation processes in the synaptic filtering of the temporal patterns of trains of action potentials. Alternatively, its inclusion within deterministic models of connections and neural networks would provide a biologically motivated challenge to the functions these might have been proposed to be performing, and help clarify the contribution made by particular cell types through these functional synaptic "timbres" that we helped uncover.

In what ways does a lack of reproducibility in signal generation provide a transmission mode that is advantageously efficient at the system level? The stochastic nature of release raises fascinating theoretical questions regarding the capacity for fast and reliable signal processing that emerges at the scale of the entire brain, particularly when it is placed in the light of
energy economics under evolutionary pressure (Attwell and Laughlin, 2001; Levy and Baxter, 2002; Laughlin and Sejnowski, 2003).

Progress in the identification of the subcellular components of the release machinery and synaptic dynamics have, as yet, gathered mostly parallel understandings at most central synapses. The elucidation of such pre- and post-synaptic conundrums can now involve combinations of advanced experimental techniques allowing visualisation and stimulation at the scale of synaptic contacts, such as two-photon microscopy and uncaging.
5.0 APPENDIX

In this section, complementary outcomes of the analysis are provided for a subset of connections, regarding the discriminatory power of the release failure identification procedure used throughout this study, and the potential impact of noise variance.

5.1 IDENTIFICATION OF RELEASE FAILURES

Failures of release were identified by eye, when no coincident spontaneous perturbation was apparent and the characteristics of the average single EPSP (baseline, rise, peak, decay) could only be matched superimposed to a single sweep voltage trace through rescaling with a null factor. Figure 5.1 shows the average traces of sweeps for which no EPSP could be identified (red) at 4 cortical connections, superimposed are the traces of the average single EPSP (black).

5.2 AMPLITUDE HISTOGRAMS

The distributions of amplitudes obtained by an automatic measurement procedure for the baseline (black), manually identified failures of release (blue), and manually identified EPSPs (red) for 4 cortical connections are shown in figure 5.2.

Following Sayer et al. (1989), time windows were defined that encompassed the peak of the average EPSP (1-2 ms long, i.e. 10-20 points) on one hand and a longer one (30-50 points) at the baseline right before the action potential used as an aligning trigger. The average difference in potential was calculated over both windows on the postsynaptic trace of every sweep. For
Figure 5.1: Average traces of manually selected release failures. The spike triggered average trace of sweeps that were indentified as including a postsynaptic potential (black) or none (red) are shown superimposed for 4 cortical connections (A-D, bottom panels). Top panels show an example of the presynaptic spike. Small schematics are included as inserts for reference to the individual connections.
each sweep the measurement then was the difference between these two values. Noise distributions were obtained in a similar manner. Identical time windows separated by the same period but located at most 50 ms before the above defined baseline time window, provided the average values of potential difference.

In all cases, the distribution of amplitudes for identified release failures was largely superimposed on the noise distribution, while the distribution of values measured for identified EPSPs was not, with essentially more of more positive values.

When overlapping portions of the failures and non-failures distributions were studied in more details, the average traces of the corresponding sweeps presented specific artefacts (data not shown). The identified release failures with the largest peak amplitude thus appeared to be those where, by chance, the values over the baseline time window were specifically lower than the baseline and those of the “peak” window were specifically higher than the baseline, whereas no indication of a rising nor decaying phase could be found. Similarly, identified EPSPs with the lowest (or even negative) peak amplitude appeared to be those where, by chance, the values over the baseline time window were specifically higher than the baseline and those of the peak window were specifically lower than the average shape would predict (time delimited notch), whereas a rising and decaying phase could still be seen. Such artefacts can sometimes be compensated for when manually measuring EPSP amplitude by scaling the average single EPSP trace on individual sweeps.
Figure 5.2: Amplitude histograms of manually selected EPSPs, release failures and noise. The difference in average postsynaptic potential measured between the time of peak and baseline preceding the presynaptic action potential is reported for sweeps that were identified as including a postsynaptic potential (red) or none (blue) for 4 cortical connections (A-D). Noise was measured identically on a portion of the traces immediately preceding the aforementioned baseline. Small schematics are included as inserts for reference to the individual connections.
5.3 FLUCTUATION ANALYSIS WITH NOISE VARIANCE SUBTRACTED

The outcomes of applying fluctuation analysis to a subset of connections where the noise variance was measured and subtracted from the values of variability in the selected subsets of amplitudes are detailed in figures 5.3 to 5.7.

For each connection noise variance was measured as the variance in the automatically measured difference in average potential from the noise measuring time windows defined in section 5.2. In this subset of connections, noise variance ranged between 0.09 and 0.13 mV^2.

Across this sample, the results provided by the fitting methods (see section 2.4.3) were impaired in one occasion (Figure 5.5), where many subsets whose variance was negative after subtraction had to be discarded from further analysis.

Mainly, the estimates provided were qualitatively similar to those provided without subtracting the noise variance: the number of functional release sites \( n \) was within one or two units of the original estimates, and only those estimates of the mean release probability \( p \) previously indicated as within the higher third of the range appeared to be consistently different but stayed within that range (Figure 5.4 and 5.6). Quantal amplitude \( q \) appeared to be the binomial parameter whose estimates were more consistently affected by noise variance subtraction. Across the sample, these estimates appeared to have lower values when the fitting methods were applied on subsets of amplitudes for which noise variance had been subtracted.

These observations are consistent with noise variance subtraction acting as a scaling factor of variability measures when applied to subsets with a limited range of mean amplitude. Provided similar levels of noise across the recordings analysed, the differences between connection classes might be expected to hold
around shifted values of the estimates, \( q \) in particular, upon systematic noise variance subtraction.
Figure 5.3: Fluctuation analysis estimating n and q applied with or without noise variance subtracted, at a layer 2 pyramid-pyramid connection. The CV^2 plot (A1, B1), CV against M (A2, B2), Failure rate against M (A3, B3), Variance against M (A4, B4) and Variance/M against M (A5, B5) are shown for the original analysis (A1-A5, see figure 3.35) and when the noise variance was subtracted from the measured variance of EPSP amplitude (B1-B5). Estimates obtained for n, q and initial release probability (p), and the corresponding coefficient of determination (r^2) are given as inserts. A schematic serves as reference to the individual connection analysed.
Figure 5.4: Fluctuation analysis estimating \( n \) and \( q \) applied with or without noise variance subtracted, at a layer 4 pyramid-pyramid connection. The CV² plot (A1, B1), CV against M (A2, B2), Failure rate against M (A3, B3), Variance against M (A4, B4) and Variance/M against M (A5, B5) are shown for the original analysis (A1-A5, see figure 3.10 and 3.11) and when the noise variance was subtracted from the measured variance of EPSP amplitude (B1-B5). Estimates obtained for \( n \), \( q \) and initial release probability (\( p \)), and the corresponding coefficient of determination (\( r^2 \)) are given as inserts. A schematic serves as reference to the individual connection analysed.
Figure 5.5: Fluctuation analysis estimating $n$ and $q$ applied with or without noise variance subtracted, at a layer 4 pyramid-pyramid connection. The CV$^2$ plot (A1, B1), CV against M (A2, B2), Failure rate against M (A3, B3), Variance against M (A4, B4) and Variance/M against M (A5, B5) are shown for the original analysis (A1-A5, see figure 3.40) and when the noise variance was subtracted from the measured variance of EPSP amplitude (B1-B5). Estimates obtained for $n$, $q$ and initial release probability ($p$), and the corresponding coefficient of determination ($r^2$) are given as inserts. A schematic serves as reference to the individual connection analysed.
Figure 5.6: Fluctuation analysis estimating $n$ and $q$ applied with or without noise variance subtracted, at a layer 5 pyramid-pyramid connection. The CV$^2$ plot (A1, B1), CV against M (A2, B2), Failure rate against M (A3, B3), Variance against M (A4, B4) and Variance/M against M (A5, B5) are shown for the original analysis (A1-A5, see figure 3.42) and when the noise variance was subtracted from the measured variance of EPSP amplitude (B1-B5). Estimates obtained for $n$, $q$ and initial release probability ($p$), and the corresponding coefficient of determination ($r^2$) are given as inserts. A schematic serves as reference to the individual connection analysed.
Figure 5.7: Fluctuation analysis estimating $n$ and $q$ applied with or without noise variance subtracted, at a layer 6 pyramid-pyramid connection. The CV$^2$ plot (A1, B1), CV against M (A2, B2), Failure rate against M (A3, B3), Variance against M (A4, B4) and Variance/M against M (A5, B5) are shown for the original analysis (A1-A5, see figure 3.46) and when the noise variance was subtracted from the measured variance of EPSP amplitude (B1-B5). Estimates obtained for $n$, $q$ and initial release probability ($p$), and the corresponding coefficient of determination ($r^2$) are given as inserts. A schematic serves as reference to the individual connection analysed.
6.0 REFERENCES


