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University College London transmission,Lecture delivered 5 October 2000 at receptor and the structural basis of fast synaptic The Croonian Lecture 2000. Nicotinic acetylcholine

Nigel Unwin

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The Croonian Lecture 2000. Nicotinic** The Croonian Lecture 2000. Nicotinic
acetylcholine receptor and the structural **he Croonian Lecture 2000. Nicotinic
Itylcholine receptor and the structural
basis of fast synaptic transmission**

Lecture delivered 5 October 2000 at University College London **Comparison Comparison Comparison Nigel Unwin**

**Migel Unwin
***Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 20H, UK*

Communication in the nervous system takes place at chemical and electrical synapses, where neurotransmitter-gated ion channels, such as the nicotinic acetylcholine (ACh) receptor, and gap junction channels control propagation of electrical signals from one cell to the next. Newly developed electron crysneurotransmitter-gated ion channels, such as the nicotinic acetylcholine (ACh) receptor, and gap junction
channels control propagation of electrical signals from one cell to the next. Newly developed electron crys-
tallogr channels control propagation of electrical signals from one cell to the next. Newly developed electron crystallographic methods have revealed the structures of these channels trapped in open as well as closed states, sugge tallographic methods have revealed the structures of these channels trapped in open as well as closed states,
suggesting how they work. The ACh receptor has large vestibules extending from the membrane-
shape the ACh-bindi suggesting how they work. The ACh receptor has large vestibules extending from the membrane which
shape the ACh-binding pockets and facilitate selective transport of cations across a narrow membrane-
spanning pore. When AC shape the ACh-binding pockets and facilitate selective transport of cations across a narrow membrane-
spanning pore. When ACh enters the pockets it triggers a concerted conformational change that opens the
pore-ly destabil spanning pore. When ACh enters the pockets it triggers a concerted conformational change that opens the
pore-by destabilizing a gate in the middle of the membrane made by a ring of pore-lining α -helical
segments. The a pore by destabilizing a gate in the middle of the membrane made by a ring of pore-lining α -helical segments. The alternative 'open' configuration of pore-lining segments reshapes the lumen and creates new surfaces, all segments. The alternative 'open' configuration of pore-lining segments reshapes the lumen and creates new surfaces, allowing the ions to pass through. The gap junction channel uses a similar structural mechanism, involvi involving coordinated rearrangements of α -helical segments in the plane of the membrane, to open its pore.

1. INTRODUCTION

1. **INTRODUCTION**
Almost 40 years ago Sir Bernard Katz delivered a
Croonian lecture on a topic related to my own The title ERT INTRODUCTION
Croonian lecture on a topic related to my own. The title
of his talk was 'The transmission of impulses from nerve Almost 40 years ago Sir Bernard Katz delivered a
Croonian lecture on a topic related to my own. The title
of his talk was 'The transmission of impulses from nerve
to muscle, and the subcellular unit of synantic action' Croonian lecture on a topic related to my own. The title
of his talk was 'The transmission of impulses from nerve
to muscle, and the subcellular unit of synaptic action'
 $(Katz 1962)$. He described a series of electrophysio of his talk was 'The transmission of impulses from nerve
to muscle, and the subcellular unit of synaptic action'
(Katz 1962). He described a series of electrophysiological
experiments in which he had dissected the process to muscle, and the subcellular unit of synaptic action'
(Katz 1962). He described a series of electrophysiological
experiments in which he had dissected the process of
rapid chemical communication, between nerve and (Katz 1962). He described a series of electrophysiological experiments in which he had dissected the process of rapid chemical communication between nerve and muscle into a number of senarable steps. That is reduction experiments in which he had dissected the process of rapid chemical communication between nerve and muscle into a number of separable steps. That is reduction rapid chemical communication between nerve and
muscle into a number of separable steps. That is reduction
of the resting potential of the nerve terminal membrane;
release in discrete 'quanta' of the neurotransmitter acetyl muscle into a number of separable steps. That is reduction
of the resting potential of the nerve terminal membrane;
release in discrete 'quanta' of the neurotransmitter acetyl-
choline (ACh): diffusion of ACh across the sy of the resting potential of the nerve terminal membrane;
release in discrete 'quanta' of the neurotransmitter acetyl-
choline (ACh); diffusion of ACh across the synaptic cleft;
hinding of ACh to receptors in the postsumpti release in discrete 'quanta' of the neurotransmitter acetyl-
choline (ACh); diffusion of ACh across the synaptic cleft; choline (ACh); diffusion of ACh across the synaptic cleft;
binding of ACh to receptors in the postsynaptic
membrane of the muscle cell; and an increase in cation
nermeability of the postsynaptic membrane and its resulbinding of ACh to receptors in the postsynaptic
membrane of the muscle cell; and an increase in cation
permeability of the postsynaptic membrane and its resul-
tant denolarization initiating the signal for the muscle to membrane of the muscle cell; and an increase in cation
the permeability of the postsynaptic membrane and its resul-
tant depolarization, initiating the signal for the muscle to \bigcup contract. Sir Bernard ended his lecture by emphasizing tant depolarization, initiating the signal for the muscle to contract. Sir Bernard ended his lecture by emphasizing the need for a molecular visualization of the events; refer-
ring to the postsynantic membrane: 'What is t contract. Sir Bernard ended his lecture by emphasizing
the need for a molecular visualization of the events; refer-
ring to the postsynaptic membrane: `...What is the
chemical constitution of the cell surface: What are the the need for a molecular visualization of the events; refer-
ring to the postsynaptic membrane: '...What is the
chemical constitution of the cell surface; What are the
molecular properties which determine its selectivity f ring to the postsynaptic membrane: \ldots What is the chemical constitution of the cell surface; What are the molecular properties which determine its selectivity for ions and how are these properties altered when a chemical constitution of the cell surface; What are the
molecular properties which determine its selectivity for
ions, and how are these properties altered when a
substance like ACb combines with some of the membrane molecular properties which determine its selectivity for
ions, and how are these properties altered when a
substance like ACh combines with some of the membrane
molecules? ions, and how are these properties altered when a
substance like ACh combines with some of the membrane
 $\frac{1}{2}$ molecules?'
A much more detailed understanding of chemical substance like ACh combines with some of the membrane

molecules?'
A much more detailed understanding of chemical
synaptic transmission has come about since that time as a
result of major advances in electrophysiological and A much more detailed understanding of chemical
synaptic transmission has come about since that time as a
result of major advances in electrophysiological and
microscopical techniques and the remarkable progress in synaptic transmission has come about since that time as a
result of major advances in electrophysiological and
microscopical techniques, and the remarkable progress in
molecular biology. One key development in terms of result of major advances in electrophysiological and
microscopical techniques, and the remarkable progress in
molecular biology. One key development in terms of
describing the initial steps was made by John Heuser and microscopical techniques, and the remarkable progress in
molecular biology. One key development in terms of
describing the initial steps was made by John Heuser and

his collaborators when they succeeded in freezing nerve
terminals at the frog neuromuscular junction a precise his collaborators when they succeeded in freezing nerve
terminals at the frog neuromuscular junction a precise
interval after a stimulus by slamming them into a block of his collaborators when they succeeded in freezing nerve
terminals at the frog neuromuscular junction a precise
interval after a stimulus by slamming them into a block of
metal cooled by liquid belium. The freeze-fracture i terminals at the frog neuromuscular junction a precise
interval after a stimulus by slamming them into a block of
metal cooled by liquid helium. The freeze-fracture images
showed pits in the presvnantic membrane in numbers interval after a stimulus by slamming them into a block of
metal cooled by liquid helium. The freeze-fracture images
showed pits in the presynaptic membrane in numbers
which were proportional to the number of quanta metal cooled by liquid helium. The freeze-fracture images
showed pits in the presynaptic membrane in numbers
which were proportional to the number of quanta
released (Heuser et al. 1979). These results thereby firmly showed pits in the presynaptic membrane in numbers
which were proportional to the number of quanta
released (Heuser *et al.* 1979). These results thereby firmly
linked the process of vesicular fusion (exocytosis) and which were proportional to the number of quanta
released (Heuser *et al.* 1979). These results thereby firmly
linked the process of vesicular fusion (exocytosis) and
quantal neurotransmitter release released (Heuser *et al.* 1979). These
linked the process of vesicular fi
quantal neurotransmitter release.
Neurotransmitter release and the linked the process of vesicular fusion (exocytosis) and
quantal neurotransmitter release.
Neurotransmitter release and the cycling of synaptic

quantal neurotransmitter release.
Neurotransmitter release and the cycling of synaptic
vesicles continues to be a topic of intense research (for
recent reviews see Fernandez-Chacon & Sudhof 1999. Neurotransmitter release and the cycling of synaptic
vesicles continues to be a topic of intense research (for
recent reviews, see Fernandez-Chacon & Sudhof 1999;
Marsh & McMahon 1999) and it has even become vesicles continues to be a topic of intense research (for
recent reviews, see Fernandez-Chacon & Sudhof 1999;
Marsh & McMahon 1999), and it has even become
possible to image single synaptic vesicles as they move to recent reviews, see Fernandez-Chacon & Sudhof 1999; Marsh & McMahon 1999), and it has even become possible to image single synaptic vesicles as they move to the membrane and fuse there (Zenisek *et al.* 2000). Marsh & McMahon 1999), and it has even become possible to image single synaptic vesicles as they move to
the membrane and fuse there (Zenisek *et al.* 2000).
However, my main purpose in this paper is to discuss
the final step in synaptic transmission involving the the membrane and fuse there (Zenisek *et al.* 2000).
However, my main purpose in this paper is to discuss
the final step in synaptic transmission, involving the
ranid excitatory or inhibitory events occurring at the However, my main purpose in this paper is to discuss
the final step in synaptic transmission, involving the
rapid excitatory or inhibitory events occurring at the
postsupantic membrane, rather than action on the the final step in synaptic transmission, involving the
rapid excitatory or inhibitory events occurring at the
postsynaptic membrane, rather than action on the
presumantic side. Increased knowledge about neurorapid excitatory or inhibitory events occurring at the
postsynaptic membrane, rather than action on the
presynaptic side. Increased knowledge about neuro-
transmitter-gated channels, selective for positive or postsynaptic membrane, rather than action on the presynaptic side. Increased knowledge about neuro-
transmitter-gated channels, selective for positive or presynaptic side. Increased knowledge about neuro-
transmitter-gated channels, selective for positive or
negative ions, has greatly extended our concept of the
postsynaptic membrane of 40 years ago. We now know transmitter-gated channels, selective for positive or
negative ions, has greatly extended our concept of the
postsynaptic membrane of 40 years ago. We now know,
for example that there are two main groups of such ion negative ions, has greatly extended our concept of the
postsynaptic membrane of 40 years ago. We now know,
for example, that there are two main groups of such ion
channels, based on amino-acid sequence: those of the postsynaptic membrane of 40 years ago. We now know,
for example, that there are two main groups of such ion
channels, based on amino-acid sequence: those of the
ACh recentor family which include the GARA. for example, that there are two main groups of such ion channels, based on amino-acid sequence: those of the ACh receptor family, which include the GABA_A (γ -aminobutyric acid type A), glycine, 5-HT₃ (5-hydroxy-ACh receptor family, which include the $GABA_A$ (γ -
aminobutyric acid type A), glycine, 5-HT₃ (5-hydroxy-
tryptamine type 3) and neuronal ACh receptors; and
those of the glutamate receptor family: the $\triangle AMA$ aminobutyric acid type A), glycine, 5-HT₃ (5-hydroxy-
tryptamine type 3) and neuronal ACh receptors; and
those of the glutamate receptor family: the AMPA
 $(\alpha_{\text{-}}\text{amino-3-hudrox-5-methyl-4-isoxzaleronerionate})$ (a-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate), those of the glutamate receptor family: the AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazoleproprionate), kainate and NMDA (N -methyl-D-aspartate) receptors.

Also important are gap junction channels, the mediators Also important are gap junction channels, the mediators
of fast electrical synaptic transmission between
connected pairs of nerve cells. There is an enormous Also important are gap junction channels, the mediators
of fast electrical synaptic transmission between
connected pairs of nerve cells. There is an enormous
diversity of these synaptic channels arising not just from of fast electrical synaptic transmission between en
connected pairs of nerve cells. There is an enormous ne
diversity of these synaptic channels, arising not just from fo
the different members of different families but als connected pairs of nerve cells. There is an enormous net
diversity of these synaptic channels, arising not just from the
the different members of different families, but also from oc
the wide range of subunit types and dis diversity of these synaptic channels, arising not just from
the different members of different families, but also from
the wide range of subunit types and distinct subunit
combinations used by the cell the different members of differes
the wide range of subunit typ
combinations used by the cell.
In the tradition of a Groom In the wide range of subunit types and distinct subunit
combinations used by the cell.
In the tradition of a Croonian lecture, I will stress

areas of personal interest and involvement leading to our In the tradition of a Croonian lecture, I will stress
areas of personal interest and involvement leading to our
present level of understanding of structure and
mechanism Most of the experiments I will refer to have areas of personal interest and involvement leading to our
present level of understanding of structure and
mechanism. Most of the experiments I will refer to have
heen conducted on the postsynantic membranes of present level of understanding of structure and
mechanism. Most of the experiments I will refer to have
been conducted on the postsynaptic membranes of
electrocytes from the *Torhedo* electric ray Electrocytes are mechanism. Most of the experiments I will refer to have
been conducted on the postsynaptic membranes of
electrocytes from the *Torpedo* electric ray. Electrocytes are
modified muscle cells and their postsynaptic membranes been conducted on the postsynaptic membranes of
electrocytes from the *Torpedo* electric ray. Electrocytes are
modified muscle cells, and their postsynaptic membranes
resemble those of the neuromuscular iunction organizing electrocytes from the *Torpedo* electric ray. Electrocytes are
modified muscle cells, and their postsynaptic membranes
resemble those of the neuromuscular junction, organizing
into a series of folds opposite the active zon modified muscle cells, and their postsynaptic membranes
resemble those of the neuromuscular junction, organizing
into a series of folds opposite the active zones where ACh
is released (Whittaker 1992) ACh recentor ion chan resemble those of the neuromuscular junction, organizing
into a series of folds opposite the active zones where ACh
is released (Whittaker 1992). ACh receptor ion channels
and the receptor-clustering protein, rapsyn, are p into a series of folds opposite the active zones where ACh
is released (Whittaker 1992). ACh receptor ion channels
and the receptor-clustering protein, rapsyn, are present
in high concentrations at the crests of these fold is released (Whittaker 1992). ACh receptor ion channels
and the receptor-clustering protein, rapsyn, are present
in high concentrations at the crests of these folds
(Sealock *et al.* 1984). Although the chemical nerve-nerv and the receptor-clustering protein, rapsyn, are present
in high concentrations at the crests of these folds
(Sealock *et al.* 1984). Although the chemical nerve-nerve
synanses of the central and nerinheral nervous systems in high concentrations at the crests of these folds (Sealock *et al.* 1984). Although the chemical nerve-nerve synapses of the central and peripheral nervous systems use different sets of postsynaptic proteins they make u (Sealock *et al.* 1984). Although the chemical nerve-nerve synapses of the central and peripheral nervous systems use different sets of postsynaptic proteins, they make use of the same or similar principles to achieve fas synapses of the central and peripheral nervous systems
use different sets of postsynaptic proteins, they make use
of the same, or similar, principles to achieve fast synaptic
transmission. The electrocyte membranes of the use different sets of postsynaptic proteins, they make use
of the same, or similar, principles to achieve fast synaptic
transmission. The electrocyte membranes of the *Torpedo*
ray like those of the neuromuscular iunction of the same, or similar, principles to achieve fast synaptic
transmission. The electrocyte membranes of the *Torpedo*
ray, like those of the neuromuscular junction, provide in
many respects an ideal system for elucidating ray, like those of the neuromuscular junction, provide in
many respects an ideal system for elucidating these principles.

2. DEVELOPMENT OF NEW IMAGING METHODS

The freeze-fracture studies on the frog neuromuscular 2. DEVELOPMENT OF NEW IMAGING METHODS
The freeze-fracture studies on the frog neuromuscular
junction were landmark experiments because they were
the first to visualize clearly synaptic events occurring on a The freeze-fracture studies on the frog neuromuscular
junction were landmark experiments because they were
the first to visualize clearly synaptic events occurring on a
millisecond time-scale. With the freeze-fracture meth junction were landmark experiments because they were
the first to visualize clearly synaptic events occurring on a
millisecond time-scale. With the freeze-fracture method,
however freezing is used at the beginning of the s the first to visualize clearly synaptic events occurring on a
millisecond time-scale. With the freeze-fracture method, our studies of the crystalline, bacteriorhodopsin-
however, freezing is used at the beginning of the sp millisecond time-scale. With the freeze-fracture method,
however, freezing is used at the beginning of the specimen
preparation process, while at the end it is always just a
surface replica that is observed in the electron however, freezing is used at the beginning of the specimen
preparation process, while at the end it is always just a
surface replica that is observed in the electron micro-
scope. Direct examination of the specimen itself preparation process, while at the end it is always just a
surface replica that is observed in the electron micro-
scope. Direct examination of the specimen itself was
needed to learn about intact three-dimensional (3D) surface replica that is observed in the electron microscope. Direct examination of the specimen itself was
needed to learn about intact three-dimensional (3D)
structure. The first successful attempts at recording scope. Direct examination of the specimen itself was needed to learn about intact three-dimensional $(3D)$ structure. The first successful attempts at recording images from frozen biological objects were by Taylor & structure. The first successful attempts at recording electron dose were too noisy for the individual bacterio-
images from frozen biological objects were by Taylor & rhodopsin molecules to be distinguished by eye, the ave Glaeser (1976). They found that the ice-embedded images from frozen biological objects were by Taylor &
Glaeser (1976). They found that the ice-embedded
specimens were stable in the microscope vacuum when
held in a cold stage at temperatures below -100° C. But Glaeser (1976). They found that the ice-embedded
specimens were stable in the microscope vacuum when
held in a cold stage at temperatures below -100° C. But
the technology—now called cryomicroscopy—only specimens were stable in the microscope vacuum when
held in a cold stage at temperatures below -100° C. But
the technology—now called cryomicroscopy—only
became established some time later as a result of some held in a cold stage at temperatures below -100° C. But
the technology—now called cryomicroscopy—only
became established some time later, as a result of some
simplifying improvements and the commercial manuthe technology—now called cryomicroscopy—only
became established some time later, as a result of some
simplifying improvements and the commercial manu-
facture of cold stages. Especially important were the became established some time later, as a result of some simplifying improvements and the commercial manufacture of cold stages. Especially important were the procedures of blotting the sample to obtain a thin provements and the commercial manu-

procedures of cold stages. Especially important were the

procedures of blotting the sample to obtain a thin

procedures of blotting the sample to obtain a thin

procedures of blotting facture of cold stages. Especially important were the
procedures of blotting the sample to obtain a thin
aqueous film on the microscope grid, and then plunging
the grid rapidly into liquid nitrogen-cooled ethane to procedures of blotting the sample to obtain a thin
aqueous film on the microscope grid, and then plunging
the grid rapidly into liquid nitrogen-cooled ethane to
create amorphous rather than crystalline ice (I epault aqueous film on the microscope grid, and then plunging
the grid rapidly into liquid nitrogen-cooled ethane to
create amorphous, rather than crystalline ice (Lepault
 et al 1983: Dubochet et al 1988) *ethe* grid rapidly into liquid nition-
 et al. 1983; Dubochet *et al.* 1988).
 Forembine the needs both

et al. 1983; Dubochet *et al.* 1988).
To combine the needs both to image the specimen
directly and to capture synaptic events on the milli-
second time-scale John Berriman and I modified the To combine the needs both to image the specimen
directly and to capture synaptic events on the milli-
second time-scale John Berriman and I modified the
cryo-plunger into a spray-freezing device (Berriman $\&$ directly and to capture synaptic events on the milli-
second time-scale John Berriman and I modified the
cryo-plunger into a spray-freezing device (Berriman &
Unwin 1994) An atomizer spray gun is activated the second time-scale John Berriman and I modified the $\frac{1}{2}$ cryo-plunger into a spray-freezing device (Berriman & Unwin 1994). An atomizer spray gun is activated the cryo-plunger into a spray-freezing device (Berriman & Unwin 1994). An atomizer spray gun is activated the moment the microscope grid, held in the plunger, is released so that a burst of spray droplets containing Unwin 1994). An atomizer spray gun is activated the
moment the microscope grid, held in the plunger, is
released, so that a burst of spray droplets, containing
ACb and ferritin marker particles impinge on the grid moment the microscope grid, held in the plunger, is
released, so that a burst of spray droplets, containing
ACh and ferritin marker particles, impinge on the grid
just $(c\sigma, 5 \text{ ms})$ before it hits the ethane surface. In t released, so that a burst of spray droplets, containing
ACh and ferritin marker particles, impinge on the grid
just (*ca*. 5 ms) before it hits the ethane surface. In this
way, the ACh in the droplets can be mixed with the

containing postsynaptic membranes over an interval long enough to open the channels, while ensuring that a containing postsynaptic membranes over an interval long
enough to open the channels, while ensuring that a
negligible fraction of them convert to a desensitized
form. Rapid dilution of the impacted spray droplets enough to open the channels, while ensuring that a
negligible fraction of them convert to a desensitized
form. Rapid dilution of the impacted spray droplets
occurs by surface spreading and diffusion but the final negligible fraction of them convert to a desensitized
form. Rapid dilution of the impacted spray droplets
occurs by surface spreading and diffusion, but the final
concentration of ACh in the vicinity of the specimen can form. Rapid dilution of the impacted spray droplets
occurs by surface spreading and diffusion, but the final
concentration of ACh in the vicinity of the specimen can
be estimated from the image by the number of ferritin occurs by surface spreading and diffusion, but the final
concentration of ACh in the vicinity of the specimen can
be estimated from the image by the number of ferritin
particles present. The ethane immersion achieves nearl concentration of ACh in the vicinity of the specimen can
be estimated from the image by the number of ferritin
particles present. The ethane immersion achieves nearly
instantaneous tranning of the reaction because the be estimated from the image by the number of ferritin
particles present. The ethane immersion achieves nearly
instantaneous trapping of the reaction because the
freezing rate $(ca, 10^6 \degree C \degree C^{-1})$ is very fast particles present. The ethane immersion
instantaneous trapping of the reactio
freezing rate (*ca.* $10^{6} °C s^{-1}$) is very fast.

3. THREE-DIMENSIONAL STRUCTURES MENSIONAL STRU
FROM IMAGES

FROM IMAGES
In addition to the requirements of directly viewing the FROM IMAGES
In addition to the requirements of directly viewing the
specimen, and of capturing transient structural changes,
methods had to be developed for extracting 3D infor-In addition to the requirements of directly viewing the
specimen, and of capturing transient structural changes,
methods had to be developed for extracting 3D infor-
mation from the electron micrographs. The contrast specimen, and of capturing transient structural changes,
methods had to be developed for extracting 3D infor-
mation from the electron micrographs. The contrast
generated by electrons interacting with organic matter is methods had to be developed for extracting 3D information from the electron micrographs. The contrast
generated by electrons interacting with organic matter is
extremely weak Eurthermore, biological molecules are generated by electrons interacting with organic matter is extremely weak. Furthermore, biological molecules are generated by electrons interacting with organic matter is
extremely weak. Furthermore, biological molecules are
highly sensitive to electron damage, and fall apart at
doses far smaller than those used in routine observatio extremely weak. Furthermore, biological molecules are
highly sensitive to electron damage, and fall apart at
doses far smaller than those used in routine observations
of stained samples. If the images are recorded with a d highly sensitive to electron damage, and fall apart at
doses far smaller than those used in routine observations
of stained samples. If the images are recorded with a dose
small enough to avoid damage, they will be too noi doses far smaller than those used in routine observations
of stained samples. If the images are recorded with a dose
small enough to avoid damage, they will be too noisy to show fine structure with clear definition. It had longth to avoid damage, they will be too noisy to
ow fine structure with clear definition.
It had long been recognized that it should be possible
surmount this statistical deficiency by averaging the

show fine structure with clear definition.
It had long been recognized that it should be possible
to surmount this statistical deficiency by averaging the
details contained in large numbers of identical molecules It had long been recognized that it should be possible
to surmount this statistical deficiency by averaging the
details contained in large numbers of identical molecules
(McLachlan 1958) Genuine features common to each to surmount this statistical deficiency by averaging the
details contained in large numbers of identical molecules
(McLachlan 1958). Genuine features common to each
molecule would be reinforced by the averaging while the details contained in large numbers of identical molecules
(McLachlan 1958). Genuine features common to each
molecule would be reinforced by the averaging, while the
fluctuations associated with electron noise (which differ (McLachlan 1958). Genuine features common to each molecule would be reinforced by the averaging, while the fluctuations associated with electron noise (which differ from one molecule to the next) would be smeared out molecule would be reinforced by the averaging, while the
fluctuations associated with electron noise (which differ
from one molecule to the next) would be smeared out.
Richard Henderson and I made use of this principle in fluctuations associated with electron noise (which differ
from one molecule to the next) would be smeared out.
Richard Henderson and I made use of this principle in
our studies of the crystalline bacteriorhodopsinfrom one molecule to the next) would be smeared out. Richard Henderson and I made use of this principle in our studies of the crystalline, bacteriorhodopsin-
containing purple membranes (Unwin & Henderson
1975), using the water-substituting medium, glucose, to
retain the native structure of the membrane in the microcontaining purple membranes (Unwin & Henderson
1975), using the water-substituting medium, glucose, to
retain the native structure of the membrane in the micro-
scone vacuum. Although micrographs of the glucose-1975), using the water-substituting medium, glucose, to retain the native structure of the membrane in the microscope vacuum. Although micrographs of the glucose-embedded membranes recorded with an appropriate low retain the native structure of the membrane in the microscope vacuum. Although micrographs of the glucose-
embedded membranes recorded with an appropriate low
electron dose were too poisy for the individual bacterioscope vacuum. Although micrographs of the glucoseembedded membranes recorded with an appropriate low aging of large numbers of identical molecules enabled us rhodopsin molecules to be distinguished by eye, the averaging of large numbers of identical molecules enabled us
to reveal the arrangement of the seven α -helical segments
crossing the linid bilayer aging of large numbers of
to reveal the arrangement
crossing the lipid bilayer.
We accomplished the ave reveal the arrangement of the seven α -helical segments
ossing the lipid bilayer.
We accomplished the averaging by densitometering the
icrographs to convert them into arrays of optical den-

Correct amorphous, rather than crystalline ice (Lepault a lattice) from the noise (which lies in-between these *et al.* 1983; Dubochet *et al.* 1988).
To combine the needs both to image the specimen obtainable by superimpo crossing the lipid bilayer.
We accomplished the averaging by densitometering the
micrographs to convert them into arrays of optical den-
sities and analysing the arrays by the computational We accomplished the averaging by densitometering the
micrographs to convert them into arrays of optical densities, and analysing the arrays by the computational
Fourier method developed for reconstructing 3D strucmicrographs to convert them into arrays of optical densities, and analysing the arrays by the computational
Fourier method developed for reconstructing 3D struc-
tures from negatively stained macromolecules (DeRosies sities, and analysing the arrays by the computational
Fourier method developed for reconstructing 3D structures from negatively stained macromolecules (DeRosier
& Klug 1968) The Fourier transform of the image of a Fourier method developed for reconstructing 3D structures from negatively stained macromolecules (DeRosier & Klug 1968). The Fourier transform of the image of a crystal separates the signal (which is at discrete points on tures from negatively stained macromolecules (DeRosier & Klug 1968). The Fourier transform of the image of a crystal separates the signal (which is at discrete points on a lattice) from the poise (which lies in-between the & Klug 1968). The Fourier transform of the image of a
crystal separates the signal (which is at discrete points on
a lattice) from the noise (which lies in-between these
points) and thereby achieves a result equivalent to crystal separates the signal (which is at discrete points on a lattice) from the noise (which lies in-between these
points) and thereby achieves a result equivalent to that
obtainable by superimposing the molecules one at a time
and averaging them in 'real space' However, the Fourie points) and thereby achieves a result equivalent to that obtainable by superimposing the molecules one at a time and averaging them in 'real space'. However, the Fourier approach in which the object is decomposed into its obtainable by superimposing the molecules one at a time
and averaging them in 'real space'. However, the Fourier
approach, in which the object is decomposed into its sine
wave components each having a distinct amplitude an and averaging them in 'real space'. However, the Fourier approach, in which the object is decomposed into its sine wave components, each having a distinct amplitude and approach, in which the object is decomposed into its sine wave components, each having a distinct amplitude and phase, is more adaptable to rapid computation and quantitative evaluation, and can more readily be extended to wave components, each having a distinct amplitude and
phase, is more adaptable to rapid computation and quan-
titative evaluation, and can more readily be extended to
the analysis of 3D structures phase, is more adaptable to rapitative evaluation, and can m
the analysis of 3D structures.
How is 2.3D structure the analysis of 3D structures.
How is a 3D structure determined from a two-

ACh and ferritin marker particles, impinge on the grid μ How is a 3D structure determined from a two-
just (*ca*. 5 ms) before it hits the ethane surface. In this dimensional (2D) crystal, such as purple membrane,
way, the analysis of 3D structures.
How is a 3D structure determined from a two-
dimensional (2D) crystal, such as purple membrane,
when an image displays only a single view—a projection How is a 3D structure determined from a two-
dimensional (2D) crystal, such as purple membrane,
when an image displays only a single view—a projection

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Figure 1. ACh receptor tubes are imaged in thin films of amorphous ice over holes in the carbon support film. (*a*) Low Figure 1. ACh receptor tubes are imaged in thin films of amorphous ice over holes in the carbon support film. (*a*) Low
magnification image, (*b*) high magnification image, showing receptors face-on near the middle of the Figure 1. ACh receptor tubes are imaged in thin films of amorphous ice over holes in the carbon support film. (a) Low
magnification image, (b) high magnification image, showing receptors face-on near the middle of the tub magnification image, (b) high magnification image, showing receptors face-on near the middle of the tube, and side-on at the edges. To obtain receptors in the open-channel form, ACh-containing droplets are sprayed onto th edges. To obtain receptors in the open-channel form, ACh-containin
the tubes *ca*. 5 ms before the rapid freezing. Ferritin marker particles
have been reacted with ACh. Scale bars, *(a)* 2000Å and *(b)* 200Å.

of the densities ? The answer is to obtain a number of different views and to combine them, making use of the property that each corresponds to a different central different views and to combine them, making use of the
property that each corresponds to a different central re
section through the 3D Fourier transform (DeRosier & M
Klug 1968: Henderson & Unwin 1975) Once this Fourier property that each corresponds to a different central
section through the 3D Fourier transform (DeRosier &
Klug 1968; Henderson & Unwin 1975). Once this Fourier
transform, has been sampled by the different central section through the 3D Fourier transform (DeRosier &
Klug 1968; Henderson & Unwin 1975). Once this Fourier
transform has been sampled by the different central
sections at a sufficient number of points to trace accu-Klug 1968; Henderson & Unwin 1975). Once this Fourier
transform has been sampled by the different central
sections at a sufficient number of points to trace accu-
rately the amplitude and phase variations, the structure transform has been sampled by the different central
sections at a sufficient number of points to trace accu-
rately the amplitude and phase variations, the structure
can be calculated by a 3D Fourier synthesis sections at a sufficient number of points to trace accurately the amplitude and phase variations, the structure can be calculated by a 3D Fourier synthesis.
Tubular crystals form the basis of almost all quantita- \Box rately the amplitude and phase variations, the structure

the structural studies of positive structural studies of postsynaptic membranes. Tubes
tive structural studies of postsynaptic membranes. Tubes
differ most obviously from 2D crystals in that their Tubular crystals form the basis of almost all quantitative structural studies of postsynaptic membranes. Tubes differ most obviously from 2D crystals in that their lattice instead of being planar, makes a continuous tive structural studies of postsynaptic membranes. Tubes
differ most obviously from 2D crystals in that their
lattice, instead of being planar, makes a continuous
network around the surface of a cylinder. For structure differ most obviously from 2D crystals in that their
lattice, instead of being planar, makes a continuous
network around the surface of a cylinder. For structure
analysis they are most usefully thought of as one-dimenlattice, instead of being planar, makes a continuous
network around the surface of a cylinder. For structure
analysis, they are most usefully thought of as one-dimen-
sional crystals (giving rise to regularly spaced layernetwork around the surface of a cylinder. For structure
analysis, they are most usefully thought of as one-dimen-
sional crystals (giving rise to regularly spaced layer-lines,
rather than spots, in their Fourier transforms analysis, they are most usefully thought of as one-dimensional crystals (giving rise to regularly spaced layer-lines, rather than spots, in their Fourier transforms), and as objects built up from helical density waves (Kl sional crystals (giving rise to regularly spaced layer-lines, rather than spots, in their Fourier transforms), and as objects built up from helical density waves (Klug *et al.* 1958; Moody 1990). Thus the layer-lines each rather than spots, in their Fourier transforms), and as objects built up from helical density waves (Klug *et al.* 1958; Moody 1990). Thus the layer-lines each give information about a particular set of helical waves, the number around the circumference of which is a property 1958; Moody 1990). Thus the layer-lines each give information about a particular set of helical waves, the number around the circumference of which is a property of the helical symmetry present. The measured applimation about a particular set of helical waves, the
number around the circumference of which is a property
of the helical symmetry present. The measured ampli-
tudes and phases along the layer-lines allow the number around the circumference of which is a property
of the helical symmetry present. The measured ampli-
tudes and phases along the layer-lines allow the

Phil. Trans. R. Soc. Lond. B (2000)

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azimuthal variation in strength and relative positions of these waves to be calculated, and hence provide all that is required to derive the average 3D structure (DeRosier & these waves to be calculated, and hence provide all that is
required to derive the average 3D structure (DeRosier &
Moore 1970). This property of a tubular crystal, arising
from its helical construction, makes it unnecessa required to derive the average 3D structure (DeRosier & Moore 1970). This property of a tubular crystal, arising
from its helical construction, makes it unnecessary to
carry out tilting experiments as needed to acquire Moore 1970). This property of a tubular crystal, arising
from its helical construction, makes it unnecessary to
carry out tilting experiments, as needed to acquire
different views with a 2D crystal. But the principle of from its helical construction, makes it unnecessary to
carry out tilting experiments, as needed to acquire
different views with a 2D crystal. But the principle of
averaging over many identical units to achieve a high carry out tilting experiments, as needed to acquire
different views with a 2D crystal. But the principle of
averaging over many identical units to achieve a high
signal-to-noise ratio is the same different views with a 2D crystal. But the principle of averaging over many identical units to achieve a high signal-to-noise ratio is the same.

4. PROPERTIES OF ACh RECEPTOR TUBES

4. PROPERTIES OF ACH RECEPTOR TUBES
A special feature of the electrocytes is the dense,
trially crystalline packing of ACh receptors on their 4. **PROPERTIES OF ACH RECEPTOR TOBES**
A special feature of the electrocytes is the dense,
partially crystalline packing of ACh receptors on their
innervated faces. Freeze-fracture photographs show the A special feature of the electrocytes is the dense,
partially crystalline packing of ACh receptors on their
innervated faces. Freeze-fracture photographs show the
receptors on the membrane surface to be grouned as partially crystalline packing of ACh receptors on their
innervated faces. Freeze-fracture photographs show the
receptors on the membrane surface to be grouped as
dimers and organized as paired ribbons packed tightly innervated faces. Freeze-fracture photographs show the receptors on the membrane surface to be grouped as dimers, and organized as paired ribbons packed tightly receptors on the membrane surface to be grouped as
dimers, and organized as paired ribbons packed tightly
side-by-side (Heuser & Salpeter 1979). Tubes are also
composed of tightly packed ribbons of receptor dimers dimers, and organized as paired ribbons packed tightly
side-by-side (Heuser & Salpeter 1979). Tubes are also
composed of tightly packed ribbons of receptor dimers
(Brisson & Unwin 1984), and grow paturally from the side-by-side (Heuser & Salpeter 1979). Tubes are also
composed of tightly packed ribbons of receptor dimers
(Brisson & Unwin 1984), and grow naturally from the
isolated electrocyte membranes (Kubalek *et al.* 1987) composed of tightly packed ribbons of receptor dimers
(Brisson & Unwin 1984), and grow naturally from the
isolated electrocyte membranes (Kubalek *et al.* 1987),
retaining a similar curvature as the crests of the (Brisson & Unwin 1984), and grow naturally from the isolated electrocyte membranes (Kubalek *et al.* 1987), retaining a similar curvature as the crests of the innectional folds. Clearly there is a close structural isolated electrocyte membranes (Kubalek *et al.* 1987), retaining a similar curvature as the crests of the junctional folds. Clearly there is a close structural

correspondence between the tubes, which are simply eloncorrespondence between the tubes, which are simply elongated protein-lipid vesicles, and the receptor-rich membrane as it exists in vivo correspondence between the tubes, which are simply elongated protein-lipid vesicles, and the receptor-rich
membrane as it exists *in vivo*.
The ACh receptor, forming the surface lattice of the
tube is a model neurotransmit

membrane as it exists *in vivo*.
The ACh receptor, forming the surface lattice of the
tube, is a model neurotransmitter-gated ion channel that
has been explored intensively over the last 25 years by all The ACh receptor, forming the surface lattice of the tube, is a model neurotransmitter-gated ion channel that has been explored intensively over the last 25 years by all kinds of techniques (Hille 1992) It is a large α tube, is a model neurotransmitter-gated ion channel that
has been explored intensively over the last 25 years by all
kinds of techniques (Hille 1992). It is a large (*ca.*
290 kDa) glycoprotein composed of a ring of five s kinds of techniques (Hille 1992). It is a large $\langle ca. 290 \text{ kDa} \rangle$ glycoprotein composed of a ring of five subunits which together delineate a gated cation-selective pathway across the membrane. There are two ACh-binding 290 kDa) glycoprotein composed of a ring of five subunits
which together delineate a gated cation-selective pathway
across the membrane. There are two ACh-binding (α)
subunits having identical amino-acid sequences and which together delineate a gated cation-selective pathway
across the membrane. There are two ACh-binding (α)
subunits, having identical amino-acid sequences, and
three others $(\beta, \gamma \text{ and } \delta)$ having sequences homologous subunits, having identical amino-acid sequences, and subunits, having identical amino-acid sequences, and
three others (β , γ and δ) having sequences homologous
to the α s (42, 35 and 36% identity to α , respectively;
Popot & Changeux 1984) Each subunit has a la three others $(\beta, \gamma \text{ and } \delta)$ having sequences homologous
to the αs (42, 35 and 36% identity to α , respectively;
Popot & Changeux 1984). Each subunit has a large
extracellular N-terminal domain four predicted transto the α s (42, 35 and 36% identity to α , respectively;
Popot & Changeux 1984). Each subunit has a large
extracellular N-terminal domain, four predicted trans-
membrane regions (M1–M4) and an extended Popot & Changeux 1984). Each subunit has a large
extracellular N-terminal domain, four predicted trans-
membrane regions $(M1-M4)$, and an extended
extends mic $M3-M4$ loop. In evolutionary terms the extracellular N-terminal domain, four predicted trans-
membrane regions $(Ml-M4)$, and an extended
cytoplasmic $M3-M4$ loop. In evolutionary terms, the
B-subunit diverged first from the α -subunit along a membrane regions (M1–M4), and an extended
cytoplasmic M3–M4 loop. In evolutionary terms, the
 β -subunit diverged first from the α -subunit, along a
senarate path from the α/δ lineage (Kubo *et al* 1985) cytoplasmic M3-M4 loop. In evolutionary terms, the β -subunit diverged first from the α -subunit, along a separate path from the γ/δ lineage (Kubo *et al.* 1985), suggesting it has a distinct functional role. The separate path from the γ/δ lineage (Kubo *et al.* 1985), subunits influence most strongly the ACh-binding propersuggesting it has a distinct functional role. The γ - and δ -
subunits influence most strongly the ACh-binding proper-
ties of the α -subunits. The δ -subunit of the *Torpedo*
receptor has near its C-terminus a cy subunits influence most strongly the ACh-binding proper-
ties of the α -subunits. The δ -subunit of the *Torpedo*
receptor has near its C-terminus a cysteine residue,
which forms intermolecular $\delta \tilde{\delta}$ disulphide b ties of the α -subunits. The δ -subunit of the *Torpedo*
receptor has near its C-terminus a cysteine residue,
which forms intermolecular $\delta-\delta$ disulphide bridges
(Chang & Bock 1977: McCrea *et al* 1987) This covalent receptor has near its C-terminus a cysteine residue,
which forms intermolecular $\delta-\delta$ disulphide bridges
(Chang & Bock 1977; McCrea *et al.* 1987). This covalent
linkage accounts for the observed pairing of the *Terhedo* which forms intermolecular $\delta-\delta$ disulphide bridges (Chang & Bock 1977; McCrea *et al.* 1987). This covalent linkage accounts for the observed pairing of the *Torpedo* recentors since the they disnerse rapidly on exposur (Chang & Bock 1977; McCrea *et al.* 1987). This covalent linkage accounts for the observed pairing of the *Torpedo* receptors, since the they disperse rapidly on exposure to reducing agents (Brisson & Unwin 1984) linkage accounts for the observed pairing of the *Torpedo* receptors, since the they disperse rapidly on exposure to reducing agents (Brisson & Unwin 1984). Europtors, since the they disperse rapidly on exposure to
ducing agents (Brisson & Unwin 1984).
Early microscopical studies of the tubes were limited
resolution to ca 30 Å because of the flattening caused

Early microscopical studies of the tubes were limited
in resolution to *ca*. 30 Å, because of the flattening caused amount of information obtained from other kinds of
by their interaction with the carbon support film inves Early microscopical studies of the tubes were limited
in resolution to *ca*. 30 Å, because of the flattening caused
by their interaction with the carbon support film
(Kistler & Stroud 1981: Brisson & Unwin 1984, 1985: in resolution to *ca*. 30 Å, because of the flattening caused
by their interaction with the carbon support film
(Kistler & Stroud 1981; Brisson & Unwin 1984, 1985;
Mitra *et al* 1989; Kubalek *et al* 1987) However Chikash by their interaction with the carbon support film
(Kistler & Stroud 1981; Brisson & Unwin 1984, 1985;
Mitra *et al.* 1989; Kubalek *et al.* 1987). However, Chikashi
Toyoshima and I found that the tubes retained their (Kistler & Stroud 1981; Brisson & Unwin 1984, 1985; Mitra *et al.* 1989; Kubalek *et al.* 1987). However, Chikashi Toyoshima and I found that the tubes retained their cylindrical cross-section in regions of amorphous ice Mitra *et al.* 1989; Kubalek *et al.* 1987). However, Chikashi
Toyoshima and I found that the tubes retained their
cylindrical cross-section in regions of amorphous ice
where they were totally surrounded by water i.e. over Toyoshima and I found that the tubes retained their cylindrical cross-section in regions of amorphous ice
where they were totally surrounded by water, i.e. over holes in the carbon support film (figure 1). Images of where they were totally surrounded by water, i.e. over
holes in the carbon support film (figure 1). Images of
tubes from such regions, when analysed by the helical
method vielded 3D maps at 17 Å resolution (Towoshima holes in the carbon support film (figure 1). Images of
tubes from such regions, when analysed by the helical
method, yielded 3D maps at 17 Å resolution (Toyoshima
 $\&$ Linuin 1990) The improved maps showed the protein tubes from such regions, when analysed by the helical
method, yielded 3D maps at 17 Å resolution (Toyoshima
& Unwin 1990). The improved maps showed the protein
subunits to be long rods arranged around a needomethod, yielded 3D maps at 17 Å resolution (Toyoshima & Unwin 1990). The improved maps showed the protein subunits to be long rods arranged around a pseudo-& Unwin 1990). The improved maps showed the protein
subunits to be long rods arranged around a pseudo-
five-fold axis in an orientation approximately normal to
the membrane plane. The ion-conducting pathway subunits to be long rods arranged around a pseudo-
five-fold axis in an orientation approximately normal to
the membrane plane. The ion-conducting pathway,
delineated by the symmetry axis consisted of a narrow five-fold axis in an orientation approximately normal to
the membrane plane. The ion-conducting pathway,
delineated by the symmetry axis, consisted of a narrow
pore across the linid bilayer bounded by $20-25 \text{ Å}$ I the membrane plane. The ion-conducting pathway, need to be averaged, but corrections need to be made for delineated by the symmetry axis, consisted of a narrow small distortions of the tubular lattices (Beroukhim & pore ac delineated by the symmetry axis, consisted of a narrow
pore across the lipid bilayer bounded by $20-25 \text{\AA}$
diameter vestibules that extended *ca*. 65 \AA into the
synantic cleft and *ca* 20 \AA towards the inter pore across the lipid bilayer bounded by $20-25 \text{\AA}$ diameter vestibules that extended *ca*. 65 Å into the synaptic cleft and *ca*. 20 Å towards the interior of the cell (figure 2). A large centrally located mass at the diameter vestibules that extended *ca*. 65\AA into the synaptic cleft and *ca*. 20\AA towards the interior of the cell (figure 2). A large centrally located mass at the cyto-
plasmic end of the recentor was though synaptic cleft and *ca*. 20 Å towards the interior of the cell is a critical factor in the structure analysis of tubes, where \Box (figure 2). A large centrally located mass at the cyto-
plasmic end of the receptor was th (figure 2). A large centrally located mass at the cyto-
plasmic end of the receptor-vas thought to be composed
of the 43kDa receptor-clustering protein, rapsyn,
because it was not present in mans calculated from tubes plasmic end of the receptor was thought to be composed
of the 43kDa receptor-clustering protein, rapsyn,
because it was not present in maps calculated from tubes
imaged in alkaline pH (Toyoshima & Unwin 1988) of the 43 kDa receptor-clustering protein, rapsyn,
because it was not present in maps calculated from tubes
imaged in alkaline pH (Toyoshima & Unwin 1988), a
condition known to release this protein (Neubig et al. **C** because it was not present in maps calculated from tubes

imaged in alkaline pH (Toyoshima & Unwin 1988), a

condition known to release this protein (Neubig *et al.* imaged in alkaline pH (Toyoshima & Unwin 1988), a condition known to release this protein (Neubig *et al.* 1979). However, later studies (see $\S 8$) revealed that the recentor extends into this central mass. The loss in condition known to release this protein (Neubig *et al.* 1979). However, later studies (see §8) revealed that the receptor extends into this central mass. The loss in wisibility was therefore presumably due to local disor 1979). However, later studies (see $\S 8$) revealed that the receptor extends into this central mass. The loss in visibility was therefore presumably due to local disorder induced by the alkaline nH receptor extends into this central mass. The loss in visibility was therefore presumably due to local disorder $\bigcup_{n=0}^{\infty}$ induced by the alkaline pH.

5. 9 AÊ MODEL OF THE ACh RECEPTOR

A major aim of the electron microscopical studies is to s. SA MODEL OF THE ACH RECEPTOR
A major aim of the electron microscopical studies is to
see the receptor in atomic detail and so provide a 3D
chemical framework for bringing together the extensive A major aim of the electron microscopical studies is to
see the receptor in atomic detail and so provide a 3D
chemical framework for bringing together the extensive *Phil. Trans. R. Soc. Lond.* B (2000)

amount of information obtained from other kinds of
investigation But the technology for doing this has not amount of information obtained from other kinds of
investigation. But the technology for doing this has not
been available and so it has only been feasible to progress amount of information obtained from other kinds of
investigation. But the technology for doing this has not
been available and so it has only been feasible to progress
towards, this, goal, in steps. Radiation damage is a investigation. But the technology for doing this has not
been available and so it has only been feasible to progress
towards this goal in steps. Radiation damage is a
particular concern in the structure analysis of tubes. been available and so it has only been feasible to progress
towards this goal in steps. Radiation damage is a
particular concern in the structure analysis of tubes. A
tubes typically contains only $1000-3000$ regularly towards this goal in steps. Radiation damage is a
particular concern in the structure analysis of tubes. A
tube typically contains only 1000–3000 regularly
arranged receptor molecules—not enough to yield a good particular concern in the structure analysis of tubes. A
tube typically contains only $1000-3000$ regularly
arranged receptor molecules—not enough to yield a good tube typically contains only 1000–3000 regularly
arranged receptor molecules—not enough to yield a good
signal-to-noise ratio beyond *ca*. 17 Å resolution. To
improve on this more molecules need to be averaged by arranged receptor molecules—not enough to yield a good
signal-to-noise ratio beyond *ca*. 17 Å resolution. To
improve on this, more molecules need to be averaged by
combining data from different tubes. A resolution of 9 Å signal-to-noise ratio beyond *ca*. 17 Å resolution. To
improve on this, more molecules need to be averaged by
combining data from different tubes. A resolution of 9 Å
was achieved initially using data from 26 tubes (*ca* improve on this, more molecules need to be averaged by combining data from different tubes. A resolution of 9 Å was achieved initially using data from 26 tubes *(ca.* 50 000 receptors) (Unwin 1993). In going beyond 9 Å, as was achieved initially using data from 26 tubes $\langle ca. 50000 \text{ receptors} \rangle$ (Unwin 1993). In going beyond 9 Å, as I will describe later, not only do many more molecules need to be averaged but corrections need to be made for 50000 receptors) (Unwin 1993). In going beyond 9\AA , as I will describe later, not only do many more molecules I will describe later, not only do many more molecules
need to be averaged, but corrections need to be made for
small distortions of the tubular lattices (Beroukhim &
I hwin 1997), and the images must be of the bighest need to be averaged, but corrections need to be made for
small distortions of the tubular lattices (Beroukhim &
Unwin 1997), and the images must be of the highest
possible electron optical quality. The quality of the image small distortions of the tubular lattices (Beroukhim & Unwin 1997), and the images must be of the highest possible electron optical quality. The quality of the image is a critical factor in the structure analysis of tubes possible electron optical quality. The quality of the image possible electron optical quality. The quality of the image
is a critical factor in the structure analysis of tubes, where
the more accurate measurement of amplitudes from elec-
tron diffraction patterns, cannot be done. A is a critical factor in the structure analysis of tubes, where
the more accurate measurement of amplitudes from elec-
tron diffraction patterns cannot be done. All recent
recording of images have therefore been conducted i the more accurate measurement of amplitudes from electron diffraction patterns cannot be done. All recent recording of images have therefore been conducted in Japan using a 300 kV field emission microscope incomortron diffraction patterns cannot be done. All recent
recording of images have therefore been conducted in
Japan, using a 300 kV field emission microscope incorpor-
ating an exceptionally stable liquid belium-cooled recording of images have therefore been conducted in
Japan, using a 300 kV field emission microscope incorpor-
ating an exceptionally stable liquid helium-cooled
specimen stage (Fujiyoshi et al. 1991: Fujiyoshi 1998) Japan, using a 300 kV field emission microscope incorrating an exceptionally stable liquid helium-competiment stage (Fujiyoshi *et al.* 1991; Fujiyoshi 1998).
The 9 Å man revealed several unsuspected features ating an exceptionally stable liquid helium-cooled
specimen stage (Fujiyoshi *et al.* 1991; Fujiyoshi 1998).
The 9 Å map revealed several unsuspected features and

specimen stage (Fujiyoshi *et al.* 1991; Fujiyoshi 1998).
The 9 Å map revealed several unsuspected features and
gave new clues about how the receptor works. In the
extracellular portion $ca = 30 \text{ Å}$ from the membrane The 9 Å map revealed several unsuspected features and
gave new clues about how the receptor works. In the
extracellular portion, *ca*. 30 Å from the membrane
surface the two α -subunits were found to contain i gave new clues about how the receptor works. In the extracellular portion, ca . 30 Å from the membrane surface, the two α -subunits were found to contain internal cavities (figure 3). These cavities were each of ab extracellular portion, *ca*. 30 Å from the membrane surface, the two α -subunits were found to contain internal cavities (figure 3). These cavities were each of about the right size to accommodate an Δ Ch molecul surface, the two α -subunits were found to contain internal
cavities (figure 3). These cavities were each of about the
right size to accommodate an ACh molecule, suggesting
that they might be the actual pockets where AC cavities (figure 3). These cavities were each of about the right size to accommodate an ACh molecule, suggesting that they might be the actual pockets where ACh binds. The binding sites did not appear to be at the α – $\$ right size to accommodate an ACh molecule, suggesting
that they might be the actual pockets where ACh binds.
The binding sites did not appear to be at the α – γ subunit that they might be the actual pockets where ACh binds.
The binding sites did not appear to be at the $\alpha-\gamma$ subunit and $\alpha-\delta$ subunit interfaces, as was (and still is) the widely held view The binding sites c
and $\alpha-\delta$ subunit
widely held view.

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extracellular vestibule is located at the bottom of each panel.
Also surprising, in the membrane-spanning portion of Figure 3. Detail at 9 Å around cavities forming the ACh-binding pockets in the two α -subunits before (–ACh) and after
(+ACh) brief exposure to ACh. (*a*,*b*) α_s -subunits and (*c*,*d*) α_r -subunits. The three rods Figure 3. Detail at 9 Å around cavities forming the ACh-binding pockets in the two α -subunits before (-ACh) and after (+ACh) brief exposure to ACh. (*a,b*) α_5 -subunits and (*c,d*) α_7 -subunits. The three rods of (+ACh) brief exposure to ACh. (a,b) α_s -subunits and (c,d) α_γ -subunits. The three rods of density encircling the α_s -cavity (pink
traces) appear to twist around its centre (+) upon binding of ACh. The three rods o in a configuration close to that of α_{δ} with ACh bound, and do not change so much upon binding of ACh. The dotted line in α_{γ}
($-\text{ACh}$) follows a tunnel connecting the cavity to the external surroundings (see a $(-ACh)$ follows a tunnel connecting the cavity to the external
thick slabs cut through the structure in a plane normal to the r
extracellular vestibule is located at the bottom of each panel.

Also surprising, in the membrane-spanning portion of each subunit, there was only one $(\alpha$ -helical) rod clearly visible not four as predicted from bydronathy plots. The Also surprising, in the membrane-spanning portion of each subunit, there was only one $(\alpha$ -helical) rod clearly visible, not four as predicted from hydropathy plots. The single rod visible in each subunit formed the wall each subunit, there was only one $(\alpha$ -helical) rod clearly
visible, not four as predicted from hydropathy plots. The
single rod visible in each subunit formed the wall lining
the pore, and presumably corresponded to the p visible, not four as predicted from hydropathy plots. The \Box single rod visible in each subunit formed the wall lining \Box the pore, and presumably corresponded to the predicted single rod visible in each subunit formed the wall lining
the pore, and presumably corresponded to the predicted
transmembrane α -helix, M2, since several kinds of
experiment had converged to indicate that this stretch the pore, and presumably corresponded to the predicted
transmembrane α -helix, M2, since several kinds of
experiment had converged to indicate that this stretch
was exposed to the ions (Hucho et al. 1986; Giraudat et al transmembrane α -helix, M2, since several kinds of experiment had converged to indicate that this stretch was exposed to the ions (Hucho *et al.* 1986; Giraudat *et al.* 1986; Impto *et al.* 1988; I conard *et al.* 1988 Gesternment had converged to indicate that this stretch was exposed to the ions (Hucho *et al.* 1986; Giraudat *et al.* 1988; Leonard *et al.* 1988). The M2 rod was exposed to the ions (Hucho *et al.* 1986; Giraudat *et al.* 1986; Imoto *et al.* 1988; Leonard *et al.* 1988). The M2 rod did not form a straight path through the lipid bilayer, but hent near its midnoint, where it was 1986; Imoto *et al.* 1988; Leonard *et al.* 1988). The M2 rod
did not form a straight path through the lipid bilayer, but
bent near its midpoint, where it was closest to the pore
axis (forme 4a). On the lipid-facing sides did not form a straight path through the lipid bilayer, but
bent near its midpoint, where it was closest to the pore
axis (figure 4*a*). On the lipid-facing sides it was flanked
by a continuous rim of density, likely to be bent near its midpoint, where it was closest to the pore axis (figure $4a$). On the lipid-facing sides it was flanked β -sheet. by a continuous rim of density, likely to be composed of β -sheet.
A tentative alignment was able to be made between the

3D densities and the amino-acid sequence of M2. This A tentative alignment was able to be made between the 3D densities and the amino-acid sequence of M2. This alignment placed the charged groups at the end of M2 symmetrically on either side of the bilayer and a highly 3D densities and the amino-acid sequence of M2. This
alignment placed the charged groups at the end of M2
symmetrically on either side of the bilayer, and a highly
conserved leucine residue (*Terhedo* α **I** eu²⁵¹: Unw alignment placed the charged groups at the end of M2
symmetrically on either side of the bilayer, and a highly
conserved leucine residue (*Torpedo* αLeu251; Unwin 1989) *Conserved leucine residue (Torpedo &Leu251; Unwin 1989)*
Phil. Trans. R. Soc. Lond. B (2000)

at the level of the bend (figure $4b$). It was suggested that the gate of the channel might be made by the leucine sideat the level of the bend (figure $4b$). It was suggested that the gate of the channel might be made by the leucine side-
chains projecting into the pore from the bends and assoat the level of the bend (figure $4b$). It was suggested that the gate of the channel might be made by the leucine side-
chains projecting into the pore from the bends and asso-
ciating side-to-side to create a tight hydr gate of the channel might be made by the leucine side-
chains projecting into the pore from the bends and asso-
ciating side-to-side to create a tight hydrophobic barrier
that would prevent passage of the hydrated ions. chains projecting into the pore from the bends and assoiting side-to-side to create a tight hydrophobic barrier
at would prevent passage of the hydrated ions.
Mutagenesis combined with electrophysiological study
function has been a valuable approach for identifying

that would prevent passage of the hydrated ions.

Mutagenesis combined with electrophysiological study

of function has been a valuable approach for identifying

the likely roles played by individual M2 side-chains in Mutagenesis combined with electrophysiological study
of function has been a valuable approach for identifying
the likely roles played by individual M2 side-chains in
affecting ion permeation. Several such experiments have of function has been a valuable approach for identifying
the likely roles played by individual M2 side-chains in
affecting ion permeation. Several such experiments have the likely roles played by individual M2 side-chains in
affecting ion permeation. Several such experiments have
highlighted the uniqueness of the conserved leucine
residue in relation to the gating mechanism. For example affecting ion permeation. Several such experiments have
highlighted the uniqueness of the conserved leucine
residue in relation to the gating mechanism. For example,
replacement of successive leucines in the ACh receptor b highlighted the uniqueness of the conserved leucine
residue in relation to the gating mechanism. For example,
replacement of successive leucines in the ACh receptor by
serines (Labarca et al. 1995) or by threonines (Eilat residue in relation to the gating mechanism. For example,
replacement of successive leucines in the ACh receptor by
serines (Labarca *et al.* 1995) or by threonines (Filatov &
White 1995) increased markedly by uniform incr replacement of successive leucines in the ACh receptor by
serines (Labarca *et al.* 1995) or by threonines (Filatov &
White 1995) increased markedly, by uniform increments,
the opening sensitivity of the channel Replacemen serines (Labarca *et al.* 1995) or by threonines (Filatov & White 1995) increased markedly, by uniform increments, the opening sensitivity of the channel. Replacement of the White 1995) increased markedly, by uniform increments,
the opening sensitivity of the channel. Replacement of the
leucine with threonine in the $GABA_A$ receptor, an ion
channel in the same family allowed ion conduction in t the opening sensitivity of the channel. Replacement of the leucine with threonine in the $GABA_A$ receptor, an ion channel in the same family, allowed ion conduction in the absence of agonist, suggesting an almost total shif leucine with threonine in the $GABA_A$ receptor, an ion
channel in the same family, allowed ion conduction in the
absence of agonist, suggesting an almost total shift in
equilibrium towards the open-channel form (Tierney *et* channel in the same family, allowed ion conduction in the absence of agonist, suggesting an almost total shift in equilibrium towards the open-channel form (Tierney *et al.*)

Figure 4. Interpretation of the closed-channel structure at 9 Å. (*a*) Channel in profile with positions of the pore-lining M2 rods,
level of the ACh-binding pockets (arrow) and estimated limits of the lipid bilayer (dotte Figure 4. Interpretation of the closed-channel structure at 9\AA . (*a*) Channel in profile with positions of the pore-lining M2 rods, level of the ACh-binding pockets (arrow) and estimated limits of the lipid bilayer net plot of the amino-acid sequence around the segment M2 (*Torpedo* α -subunit); the cross denotes the conserved leucine residue,
L251, which may form the gate of the channel; the dots denote other residues that have b net plot of the amino-acid sequence around the segment M2 (*Torpedo* α -subunit); the cross denotes the conserved leucine residue,
L251, which may form the gate of the channel; the dots denote other residues that have be L251, which may form the gate
of an open channel blocker (Lee
Adapted from Unwin (1993).

Exampled from Enwin (1995) .

1996). However, the effect of a mutation in the functional

region of a simple protein cannot be predicted even when 1996). However, the effect of a mutation in the functional
region of a simple protein cannot be predicted even when
the atomic structure is known (e.g. Craik et al. 1985). Thus 1996). However, the effect of a mutation in the functional
region of a simple protein cannot be predicted even when
the atomic structure is known (e.g. Craik *et al.* 1985). Thus
a definitive interpretation of the role of region of a simple protein cannot be predicted even when
the atomic structure is known (e.g. Craik *et al.* 1985). Thus
a definitive interpretation of the role of the leucine is not
possible from this kind of experimental the atomic structure is known (e.g. Craik *et al.* 1985 a definitive interpretation of the role of the leucine possible from this kind of experimental approach.

6. DISTINCT CONFORMATIONS OF THE ^a**-SUBUNITS**

DISTINCT CONFORMATIONS OF THE α **-SUBUNITS**
One interesting observation made at this stage, but
t published was that the two α -subunits looked \Box One interesting observation made at this stage, but unequivocally that these subunits do have different onto published, was that the two α -subunits looked conformations in the closed-channel form of the receptor d One interesting observation made at this stage, but
not published, was that the two α -subunits looked
different, despite their amino-acid sequences being the
same. The α -cavity was more circular in cross-section not published, was that the two α -subunits looked
different, despite their amino-acid sequences being the
same. The α_{δ} -cavity was more circular in cross-section,
and the three rods of density shaning the cavities different, despite their amino-acid sequences being the
same. The α_{δ} -cavity was more circular in cross-section,
and the three rods of density shaping the cavities were
not equivalent in the two subunits (forme 3) We same. The α_{δ} -cavity was more circular in cross-section, and the three rods of density shaping the cavities were and the three rods of density shaping the cavities were
not equivalent in the two subunits (figure 3). Were their
different appearances genuine ones brought about
because these subunits might be unequally distorted by not equivalent in the two subunits (figure 3). Were their
different appearances genuine ones brought about
because these subunits might be unequally distorted by
their different sets of interactions with neighbouring different appearances genuine ones brought about
because these subunits might be unequally distorted by
their different sets of interactions with neighbouring
subunits? Or were their differences due to systematic because these subunits might be unequally distorted by their different sets of interactions with neighbouring $\frac{1}{2}$ subunits? Or were their differences due to systematic their different sets of interactions with neighbouring
subunits? Or were their differences due to systematic
errors associated with the helical method of structure
determination (which had not previously been applied to subunits? Or were their differences due to systematic
errors associated with the helical method of structure
determination (which had not previously been applied to
such high resolution)? errors associated with the
determination (which ha
such high resolution)?
The differences were determination (which had not previously been applied to
such high resolution)?
The differences were indeed genuine properties of the

receptor, as was shown by an alternative structural

approach. Occasionally it is possible to obtain 'giant' ACh
receptor tubes from the vesicle preparations. Such tubes approach. Occasionally it is possible to obtain 'giant' ACh receptor tubes from the vesicle preparations. Such tubes
are 2000–3000 Å across, and can be flattened uniformly receptor tubes from the vesicle preparations. Such tubes are $2000-3000 \text{ Å}$ across, and can be flattened uniformly receptor tubes from the vesicle preparations. Such tubes
are $2000-3000 \text{ Å}$ across, and can be flattened uniformly
on the microscope grid, making them amenable to
analysis as (overlapping) 2D crystals. The two indepenare 2000–3000 Å across, and can be flattened uniformly
on the microscope grid, making them amenable to
analysis as (overlapping) 2D crystals. The two indepen-
dently determined structures, obtained from cylinders on on the microscope grid, making them amenable to
analysis as (overlapping) 2D crystals. The two indepen-
dently determined structures, obtained from cylinders on
the one hand and planar arrays on the other showed the analysis as (overlapping) 2D crystals. The two independently determined structures, obtained from cylinders on the one hand and planar arrays on the other, showed the dently determined structures, obtained from cylinders on
the one hand and planar arrays on the other, showed the
same differences between the α -subunits, confirming
unequivocally that these subunits do have different the one hand and planar arrays on the other, showed the same differences between the α -subunits, confirming unequivocally that these subunits do have different conformations in the closed-channel form of the recentor same differences between the α -subunits, confirming
unequivocally that these subunits do have different
conformations in the closed-channel form of the receptor
(Unwin 1996) conformations in the closed-channel form of the receptor

7. CHANGES UPON ACTIVATION BY ACh

7. CHANGES UPON ACTIVATION BY ACh
The receptor channels open with remarkable speed and
iciency at the synapse, when triggered by entry of ACh r. CHANGES UPON ACTIVATION BY ACH
The receptor channels open with remarkable speed and
efficiency at the synapse, when triggered by entry of ACh
molecules into their binding pockets. They open within The receptor channels open with remarkable speed and
efficiency at the synapse, when triggered by entry of ACh
molecules into their binding pockets. They open within
about 20 us of binding and achieve a high probability of efficiency at the synapse, when triggered by entry of ACh molecules into their binding pockets. They open within about 20 μ s of binding and achieve a high probability of molecules into their binding pockets. They open within
about 20 µs of binding and achieve a high probability of
opening (*ca.* 0.95) until the ACh has been degraded by
acetylcholinesterase or until desensitization takes pl about 20 μ s of binding and achieve a high probability of
opening (ca. 0.95) until the ACh has been degraded by
acetylcholinesterase or until desensitization takes place
(Colcuboun & Sakmann 1985) Desensitization occurs opening (*ca*. 0.95) until the ACh has been degraded by
acetylcholinesterase or until desensitization takes place
(Colquhoun & Sakmann 1985). Desensitization occurs
within *ca* 20 ms, in the continued presence of ACh acetylcholinesterase or until desensitization takes place
(Colquhoun & Sakmann 1985). Desensitization occurs
within *ca*. 20 ms in the continued presence of ACh
(Matsubara et al. 1992). Thus in order to convert the (Colquhoun & Sakmann 1985). Desensitization occurs
within *ca*. 20 ms in the continued presence of ACh
(Matsubara *et al.* 1992). Thus in order to convert the

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Figure 5. ACh-induced changes at the level of the gate, in Figure 5. ACh-induced changes at the level of the gate, in
the middle of the membrane. The M2 rods are *ca.* 2 Å further
from the central axis after (full lines) than before (broken Figure 5. ACh-induced changes at the level of the gate, in
the middle of the membrane. The M2 rods are ca . 2 Å further
from the central axis after (full lines) than before (broken
lines) exposure to ACh. In contrast, the from the central axis after (full lines) than before (broken
lines) exposure to ACh. In contrast, the outer rim of density from the central axis after (full lines) than before (broken
lines) exposure to ACh. In contrast, the outer rim of densit
does not change significantly (indicated by almost exact
superposition of the two contours) lines) exposure to ACh. In contrast,
does not change significantly (indica
superposition of the two contours).

superposition of the two contours).
receptor into the open-channel form and retain this form
so that its structure can be analysed extremely brief rereceptor into the open-channel form and retain this form
so that its structure can be analysed, extremely brief re-
action with ACh is needed, followed by tranning of the receptor into the open-channel form and retain this form
so that its structure can be analysed, extremely brief re-
action with ACh is needed, followed by trapping of the
structural response so that its structure
action with ACh is r
structural response.
I described above i Fractural response.
I described above in $\S 2$ the method devised to achieve
d tran the open channel. In brief, a solution of ACh

structural response.
I described above in $\S 2$ the method devised to achieve
and trap the open channel. In brief, a solution of ACh, I described above in $\S 2$ the method devised to achieve
and trap the open channel. In brief, a solution of ACh,
containing ferritin marker particles, is sprayed onto
tubes in a thin aqueous film on the electron microscop and trap the open channel. In brief, a solution of ACh,
containing ferritin marker particles, is sprayed onto
tubes in a thin aqueous film on the electron microscope
grid and the grid is then plunged rapidly (within 5 ms) containing ferritin marker particles, is sprayed onto
tubes in a thin aqueous film on the electron microscope
grid, and the grid is then plunged rapidly (within 5 ms)
into liquid nitrogen-cooled ethane. Since a 3D structur tubes in a thin aqueous film on the electron microscope
grid, and the grid is then plunged rapidly (within 5 ms)
into liquid nitrogen-cooled ethane. Since a 3D structure
of the receptor can be derived from a single image o grid, and the grid is then plunged rapidly (within 5 ms)
into liquid nitrogen-cooled ethane. Since a 3D structure
of the receptor can be derived from a single image of a
tube it was possible to investigate systematically t into liquid nitrogen-cooled ethane. Since a 3D structure
of the receptor can be derived from a single image of a
tube, it was possible to investigate systematically the
effect of exposure to different concentrations of ACb of the receptor can be derived from a single image of a
tube, it was possible to investigate systematically the Figure 6. Transient configuration of M2 rods around the
effect of exposure to different concentrations of ACh tube, it was possible to investigate systematically the Figure 6. Transient configuration of M2 rods around the effect of exposure to different concentrations of ACh open pore, and interpretation at 9\AA . (a) A barrel effect of exposure to different concentrations of ACh reproducible structural change over concentrations used
in the data sellection (100 M, 10 mM), as would be same level as for the closed pore (see figure 4), but instead of particles in the image). I found there was a small, but
reproducible structural change over concentrations used
in the data collection $(100 \mu M - 10 \text{ mM})$, as would be
expected if there were always a high proportion of ope reproducible structural change over concentrations used
in the data collection $(100 \mu\text{M} - 10 \text{mM})$, as would be
expected if there were always a high proportion of open
channels under these conditions (Unwin 1995). On in the data collection $(100 \mu M - 10 \text{ m})$, as would be expected if there were always a high proportion of open channels under these conditions (Unwin 1995). On the other hand prolonged desensitizing exposure to ACh Expected if there were always a high proportion of open
channels under these conditions (Unwin 1995). On the representation of the most distant three rods. A tentative
other hand prolonged, desensitizing exposure to ACh
i channels under these conditions (Unwin 1995). On the other hand prolonged, desensitizing exposure to ACh gave rise to a much greater and different kind of change D change. ve rise to a much greater and different kind of
ange.
The transition from the closed to the open channel was
alveed by comparing the new map from the sprayed

change.
The transition from the closed to the open channel was
analysed by comparing the new map from the sprayed
tubes (also at 9 Å resolution, from ca 50,000 molecules) The transition from the closed to the open channel was
analysed by comparing the new map from the sprayed
tubes (also at 9 Å resolution, from ca . 50 000 molecules)
with the old. Most affected was the region around and analysed by comparing the new map from the sprayed
tubes (also at 9 Å resolution, from *ca*. 50 000 molecules)
with the old. Most affected was the region around and
close to the cavities i.e. the ACh-binding pockets. C tubes (also at 9 Å resolution, from *ca.* 50 000 molecules) with the old. Most affected was the region around and close to the cavities, i.e. the ACh-binding pockets. The with the old. Most affected was the region around and
close to the cavities, i.e. the ACh-binding pockets. The
disturbance at this level was complex, involving all five
subunits. However, it is useful to focus on the α close to the cavities, i.e. the ACh-binding pockets. The disturbance at this level was complex, involving all five subunits. However, it is useful to focus on the α -subunits, one of which $(\alpha_2$ forme $3a$ b) was alter disturbance at this level was complex, involving all five
subunits. However, it is useful to focus on the α -subunits,
one of which (α_{δ} figure 3*a*,*b*) was altered a large amount
by the interaction with ACh and t subunits. However, it is useful to focus on the α -subunits, one of which (α_{δ} figure 3*a*,*b*) was altered a large amount by the interaction with ACh, and the other of which (α_{ν}) ; figure $3c$, d) was altered less. The subunits ended up by the interaction with ACh, and the other of which $(\alpha_{\gamma};$ figure $3c,d$) was altered less. The subunits ended up looking more similar to each other, with the cavities becoming less prominent due to the fact that they no figure $3c,d$) was altered less. The subunits ended up
looking more similar to each other, with the cavities
becoming less prominent due to the fact that they now
contained ΔC b Δ superficial explanation of these looking more similar to each other, with the cavities
becoming less prominent due to the fact that they now
contained ACh. A superficial explanation of these
changes is that the α -subunits are initially distorted becoming less prominent due to the fact that they now contained ACh. A superficial explanation of these changes is that the α -subunits are initially distorted unequally by their interactions with neighbouring contained ACh. A superficial explanation of these changes is that the α -subunits are initially distorted unequally by their interactions with neighbouring an unequally by their interactions with neighbouring
Phil. Trans. R. Soc. Lond. B (2000)

Figure 6. Transient configuration of $M2$ rods around the Figure 6. Transient configuration of M2 rods around the open pore, and interpretation at 9\AA . (*a*) A barrel of α -helical segments having a pronounced twist, forms in the cytoplasmic Figure 6. Transient configuration of M2 rods around the
open pore, and interpretation at 9 Å . (a) A barrel of α -helical
segments, having a pronounced twist, forms in the cytoplasmic
leaflet of the bilayer, constri open pore, and interpretation at 9 Å . (a) A barrel of α -helical segments, having a pronounced twist, forms in the cytoplasmic
leaflet of the bilayer, constricting the pore maximally at the
cytoplasmic membrane sur segments, having a pronounced twist, forms in the cytoplasmic
leaflet of the bilayer, constricting the pore maximally at the
cytoplasmic membrane surface. The bend in the rods is at the
same level as for the closed pore (s leaflet of the bilayer, constricting the pore maximally at the cytoplasmic membrane surface. The bend in the rods is at the same level as for the closed pore (see figure 4), but instead of pointing inwards has rotated ove cytoplasmic membrane surface. The bend in the rods is at the same level as for the closed pore (see figure 4), but instead of pointing inwards has rotated over to the side. (b) Schematic representation of the most dista same level as for the closed pore (see figure 4), but instead o
pointing inwards has rotated over to the side. (*b*) Schematic
representation of the most distant three rods. A tentative
alignment of the amipo-acid sequenc pointing inwards has rotated over to the side. (b) Schemat
representation of the most distant three rods. A tentative
alignment of the amino-acid sequence with the densities
suggests that a line of polar residues (serine representation of the most distant three rods. A tentative
alignment of the amino-acid sequence with the densities
suggests that a line of polar residues (serines and threonine;
see foure 4b) should be facing the open por see figure $4b$) should be facing the open pore. (From Unwin 1995.)

 (1995)
subunits, and that the free energy derived from the
hinding of two ACh molecules is able to partially oversubunits, and that the free energy derived from the binding of two ACh molecules is able to partially over-
come these distortions, making the conformations of the subunits, and that the free energy derived from the binding of two ACh molecules is able to partially over-
come these distortions, making the conformations of the
subunits more nearly equal binding of two ACh molecules is able to partially over-
come these distortions, making the conformations of the
subunits more nearly equal.

At the time of this work, in 1995, we could not clearly subunits more nearly equal.
At the time of this work, in 1995, we could not clearly
resolve tunnels that lead into, and out of the cavities, and
therefore provide routes by which the ACh can access or At the time of this work, in 1995, we could not clearly
resolve tunnels that lead into, and out of the cavities, and
therefore provide routes by which the ACh can access or
leave the binding pockets. These tunnels, and the resolve tunnels that lead into, and out of the cavities, and
therefore provide routes by which the ACh can access of
leave the binding pockets. These tunnels, and the differ-
ences in conformation of the α -subunits arou therefore provide routes by which the ACh can access or
leave the binding pockets. These tunnels, and the differ-
ences in conformation of the α -subunits around the
pockets are central to our present understanding of h leave the binding pockets. These tunnels, and the differences in conformation of the α -subunits around the pockets, are central to our present understanding of how the recentor functions as a gated ion channel and I wi ences in conformation of the α -subunits around the pockets, are central to our present understanding of how the receptor functions as a gated ion channel, and I will describe them in some detail later pockets, are central to our present understanding of how
the receptor functions as a gated ion channel, and I will
describe them in some detail later. Exerceptor functions as a gated ion channel, and I will
scribe them in some detail later.
Comparison of the two maps also suggested that
gere were small rotations of the subunits (mainly of

describe them in some detail later.
Comparison of the two maps also suggested that
there were small rotations of the subunits (mainly of

Figure 7. Schematic drawing of the opening mechanism Figure 7. Schematic drawing of the opening mechanism
suggested by the freeze-trapping experiments. Binding of ACh
to both α -subunits initiates a concerted disturbance at the Figure 7. Schematic drawing of the opening mechanism
suggested by the freeze-trapping experiments. Binding of AC
to both α -subunits initiates a concerted disturbance at the
level of the binding pockets, which leads to suggested by the freeze-trapping experiments. Binding of ACh
to both α -subunits initiates a concerted disturbance at the
level of the binding pockets, which leads to small (clockwise)
rotations of the α -subunits at to both α -subunits initiates a concerted disturbance at the level of the binding pockets, which leads to small (clockwise) rotations of the α -subunits at the level of the membrane. The rotations destabilize the asso level of the binding pockets, which leads to small (clockwise)
rotations of the α -subunits at the level of the membrane. The
rotations destabilize the association of bent α -helices forming
the gate, and favour the a rotations of the α -subunits at the level of the membrane. The
rotations destabilize the association of bent α -helices formin
the gate, and favour the alternative mode of association
(figure 6) in which the pore is w rotations destabilize the association of bent α -helices forming
the gate, and favour the alternative mode of association
(figure 6), in which the pore is wider at the middle of the
membrane and most constricted at the the gate, and favour the alternative mode of association
(figure 6), in which the pore is wider at the middle of the
membrane and most constricted at the cytoplasmic membrane
surface (Adapted from Unwin 1998) \bigcirc (figure 6), in which the pore is wider at the middle of the membrane surface. (Adapted from Unwin 1998.)

the α -subunits) linking the disturbances around the the α -subunits) linking the disturbances around the binding pockets to the membrane-spanning part of the receptor. In the membrane, the exposure to ACb did the α -subunits) linking the disturbances around the binding pockets to the membrane, the exposure to ACh did root bring about any obvious alteration of the rim of binding pockets to the membrane-spanning part of the
receptor. In the membrane, the exposure to ACh did
not bring about any obvious alteration of the rim of
density facing the linids, whereas the M2 rods switched receptor. In the membrane, the exposure to ACh did
not bring about any obvious alteration of the rim of
density facing the lipids, whereas the M2 rods switched
quite dramatically to a new configuration in which the not bring about any obvious alteration of the rim of
density facing the lipids, whereas the M2 rods switched
quite dramatically to a new configuration in which the
hends instead of pointing towards the axis of the pore density facing the lipids, whereas the M2 rods switched
quite dramatically to a new configuration in which the
bends, instead of pointing towards the axis of the pore, quite dramatically to a new configuration in which the
bends, instead of pointing towards the axis of the pore,
had rotated over to the side. As figure 5 shows, the
pore was opened up in the middle of the membrane as bends, instead of pointing towards the axis of the pore,
had rotated over to the side. As figure 5 shows, the
pore was opened up in the middle of the membrane as
a result of this action, increasing its diameter there by had rotated over to the side. As figure 5 shows, the pore was opened up in the middle of the membrane as a result of this action, increasing its diameter there by $ca \neq \hat{A}$ pore was opened up in the middle of the membrane as
a result of this action, increasing its diameter there by
ca. 4 Å.

In the cytoplasmic leaflet, the configuration of $M2$ rods around the open pore consisted of a 'barrel' of α -helical segments (figure 6*a*), resembling the right-handed twisted around the open pore consisted of a 'barrel' of α -helical
segments (figure 6a), resembling the right-handed twisted
barrels of pore-lining α -helices found in the bacterial
toxin, B-pentamers (Merritt, & Hol. 1995), segments (figure 6a), resembling the right-handed twisted
barrels of pore-lining α -helices found in the bacterial
toxin B-pentamers (Merritt & Hol 1995) and the
mechanosensitive channel MscL (Chang et al. 1998) barrels of pore-lining α -helices found in the bacterial
toxin B-pentamers (Merritt $\&$ Hol 1995) and the
mechanosensitive channel, MscL (Chang *et al.* 1998).
However in the case of the recentor the α -helical toxin B-pentamers (Merritt & Hol 1995) and the mechanosensitive channel, MscL (Chang *et al.* 1998). mechanosensitive channel, MscL (Chang *et al.* 1998).
However, in the case of the receptor, the α -helical
arrangement gave the pore a strongly tapered shape,
making it most constricted at the evtoplasmic membrane However, in the case of the receptor, the α -helical arrangement gave the pore a strongly tapered shape, making it most constricted at the cytoplasmic membrane surface. Furthermore, only the lower portions of the α -However, in the case of the receptor, the α -helical The positions of the two α -subunits, the binding pockets arrangement gave the pore a strongly tapered shape, (asterisks), gate of the closed channel (upper arrow) surface. Furthermore, only the lower portions of the α -
helices came close enough to each other to be stabilized
by side-to-side interactions around the ring. This limited
association, combined with the rigidity of a h helices came close enough to each other to be stabilized
by side-to-side interactions around the ring. This limited
association, combined with the rigidity of a barrel, might
be important in ensuring both precise permeatio by side-to-side interactions around the ring. This limited
association, combined with the rigidity of a barrel, might
be important in ensuring both precise permeation and
fast gating kinetics association, combined
be important in ens
fast gating kinetics. important in ensuring both precise permeation and
at gating kinetics.
A tentative alignment of the densities in the cyto-
asmic leaflet with the M2 sequence suggested that a line

plast gating kinetics.

A tentative alignment of the densities in the cyto-

plasmic leaflet with the M2 sequence suggested that a line A tentative alignment of the densities in the cyto-
plasmic leaflet with the M2 sequence suggested that a line
of small polar (serine or threonine) residues would lie
almost parallel to the axis of the pore when the channe plasmic leaflet with the M2 sequence suggested that a line
of small polar (serine or threonine) residues would lie
almost parallel to the axis of the pore when the channel
opens (figure 6b) an orientation that should stab of small polar (serine or threonine) residues would lie
almost parallel to the axis of the pore when the channel
opens (figure 6*b*), an orientation that should stabilize the
passing ions by providing an environment of hig almost parallel to the axis of the pore when the channel
opens (figure $6b$), an orientation that should stabilize the
passing ions by providing an environment of high polariz-
ability. The threonine residue at the point opens (figure $6b$), an orientation that should stabilize the
passing ions by providing an environment of high polariz-
ability. The threonine residue at the point of maximum
constriction (*Tathedo* eThr⁹⁴⁴) when substi passing ions by providing an environment of high polarizability. The threonine residue at the point of maximum
constriction (*Torpedo* α Thr244), when substituted by other
residues of different volume, has a propounced e ability. The threonine residue at the point of maximum
constriction (*Torpedo* α Thr244), when substituted by other
residues of different volume, has a pronounced effect on
ion flow as if it is at the narrowest part of constriction (*Torpedo* α Thr244), when substituted by other residues of different volume, has a pronounced effect on ion flow, as if it is at the narrowest part of the open pore (Imoto, *et al.* 1991; Villarmol, *et al* residues of different volume, has a pronounced effect on
ion flow, as if it is at the narrowest part of the open pore
(Imoto *et al.* 1991; Villarroel *et al.* 1991). Mutagenesis
experiments conducted on residues in the lo ion flow, as if it is at the narrowest part of the open pore
(Imoto *et al.* 1991; Villarroel *et al.* 1991). Mutagenesis
experiments conducted on residues in the loop next to the
threonine residue also emphasize the crit (Imoto *et al.* 1991; Villarroel *et al.* 1991). Mutagenesis experiments conducted on residues in the loop next to the threonine residue also emphasize the critical nature of this region (Wilson & Karlin 1998; Corringer experiments conducted on residues in the loop next to the threonine residue also emphasize the critical nature of this region (Wilson & Karlin 1998; Corringer *et al.* 1999), although the effect of the substitutions and/or threonine residue also emphasize the critical nature of this region (Wilson & Karlin 1998; Corringer *et al.* 1999), although the effect of the substitutions and/or insertions *Phil. Trans. R. Soc. Lond.* B (2000)

Figure 8. Architecture of whole receptor, emphasizing the Figure 8. Architecture of whole receptor, emphasizing the
external surface and openings to the ion-conducting pathway
on the extracellular and cytoplasmic sides of the membrane Figure 8. Architecture of whole receptor, emphasizing the external surface and openings to the ion-conducting pathway
on the extracellular and cytoplasmic sides of the membrane.
The positions of the two α -subunits, the on the extracellular and cytoplasmic sides of the membrane. on the extracellular and cytoplasmic sides of the membrane.
The positions of the two α -subunits, the binding pockets
(asterisks), gate of the closed channel (upper arrow) and the
constriction part of the open channel (The positions of the two α -subunits, the binding pockets
(asterisks), gate of the closed channel (lower arrow) and the
constricting part of the open channel (lower arrow) are
indicated. The subunits are slightly tilted (asterisks), gate of the closed channel (upper arrow) and the constricting part of the open channel (lower arrow) are indicated. The subunits are slightly tilted around the axis of the recentor: thus it is the α -subuni constricting part of the open channel (lower arrow) are
indicated. The subunits are slightly tilted around the axis of
the receptor; thus it is the α_{δ} -subunit that can be seen through
the 'window' in the wall of the indicated. The subunits are slightly tilted around the axis of
the receptor; thus it is the α_{δ} -subunit that can be seen through
the 'window' in the wall of the cytoplasmic vestibule at the
hack of the receptor the receptor; thus it is
the 'window' in the wa
back of the receptor.

on the folding of this sensitive part of the protein are unknown. the folding of this sensitive part of the protein are
known.
A simple mechanistic picture of the structural transi-
an obtained from the study of the activated receptor was

A simple mechanistic picture of the structural transition, obtained from the study of the activated receptor, was A simple mechanistic picture of the structural transition, obtained from the study of the activated receptor, was as follows (figure 7). First, ACh triggers distinct, localized disturbances at the binding sites in the two tion, obtained from the study of the activated receptor, was
as follows (figure 7). First, ACh triggers distinct, localized
disturbances at the binding sites in the two α -subunits.
Second, the effects of these of these as follows (figure 7). First, ACh triggers distinct, localized
disturbances at the binding sites in the two α -subunits.
Second, the effects of these of these disturbances are
communicated through small rotations of the disturbances at the binding sites in the two α -subunits.
Second, the effects of these of these disturbances are
communicated, through small rotations of the α -subunits,
to the structure in the membrane Third, the M? Second, the effects of these of these disturbances are communicated, through small rotations of the α -subunits, to the structure in the membrane. Third, the M2 segments in the membrane transmit the rotations to the communicated, through small rotations of the α -subunits,
to the structure in the membrane. Third, the M2
segments in the membrane transmit the rotations to the
gate-forming side-chains, drawing them away from the to the structure in the membrane. Third, the M2
segments in the membrane transmit the rotations to the
gate-forming side-chains, drawing them away from the
central axis: the mode of association near the middle of segments in the membrane transmit the rotations to the gate-forming side-chains, drawing them away from the central axis; the mode of association near the middle of the membrane is thereby disfavoured and the segments gate-forming side-chains, drawing them away from the
central axis; the mode of association near the middle of
the membrane is thereby disfavoured, and the segments
switch to the alternative side-to-side mode of association central axis; the mode of association near the middle of
the membrane is thereby disfavoured, and the segments
switch to the alternative side-to-side mode of association,
creating an open pore the membrane is thereby disfavoured, and the segments switch to the alternative side-to-side mode of association, creating an open pore.

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8. ACh RECEPTOR AT HIGHER RESOLUTION

The resolution has been extended recently to about **EXECUTE OF AT HIGHER RESOLUTION**
The resolution has been extended recently to about
4 Å, as a result of further averaging (*ca.* 800 000
recentors) and by incorporating the technical improve-The resolution has been extended recently to about 4\AA , as a result of further averaging (*ca.* 800 000 receptors) and by incorporating the technical improve-
ments I mentioned earlier. This allows a more accurate $4 \text{ Å},$ as a result of further averaging (*ca.* 800 000 receptors) and by incorporating the technical improvements I mentioned earlier. This allows a more accurate description of the channel-opening mechanism and receptors) and by incorporating the technical improve-
ments I mentioned earlier. This allows a more accurate
description of the channel-opening mechanism, and
other properties of the receptor, than was possible when while ments I mentioned earlier. This allows a more accurate
description of the channel-opening mechanism, and
other properties of the receptor, than was possible when
the earlier (9 Å) work was published and brings the description of the channel-opening mechanism, and
other properties of the receptor, than was possible when
the earlier (9 Å) work was published, and brings the
analysis close to answering definitively some fundamental other properties of the receptor, than was possible when
the earlier (9 Å) work was published, and brings the
analysis close to answering definitively some fundamental
questions. For example, how is the cation-selecti the earlier (9 Å) work was published, and brings the
analysis close to answering definitively some fundamental
questions. For example, how is the cation-selectivity
achieved? What is the construction of the gate? How analysis close to answering definitively some fundamental
questions. For example, how is the cation-selectivity
achieved? What is the construction of the gate? How
does ACh get into (and out of) the hinding pockets? questions. For example, how is the cation-selectivity
achieved? What is the construction of the gate? How
does ACh get into (and out of) the binding pockets?
How does the binding of ACh trigger the structural achieved? What is the construction of the gate? How does ACh get into (and out of) the binding pockets?
How does the binding of ACh trigger the structural changes that disrupt the gate? does ACh get into (and out of
How does the binding of ACI
changes that disrupt the gate?
Before going into these question before the binding of ACh trigger the structural
anges that disrupt the gate?
Before going into these questions and describing some
the bigher resolution features first I should summarize

changes that disrupt the gate?
Before going into these questions and describing some
of the higher resolution features, first I should summarize
the architecture of the receptor (figure 8). The whole Before going into these questions and describing some
of the higher resolution features, first I should summarize
the architecture of the receptor (figure 8). The whole
complex is $ca = 160 \text{ Å}$ long and has pseudo-fivefol of the higher resolution features, first I should summarize
the architecture of the receptor (figure 8). The whole
complex is *ca.* 160 Å long, and has pseudo-fivefold
symmetry over its entire length except for last *ca* the architecture of the receptor (figure 8). The whole complex is *ca.* 160 Å long, and has pseudo-fivefold symmetry over its entire length, except for last *ca*. 20 Å of its cytoplasmic end which may belong to complex is *ca.* 160 Å long, and has pseudo-fivefold asymmetry over its entire length, except for last *ca.* 20 Å of this cytoplasmic end, which may belong to rapsyn (Miya-
zawa *et al.* 1999). The extracellular vestibule its cytoplasmic end, which may belong to rapsyn (Miyazawa *et al.* 1999). The extracellular vestibule is a *ca*. 65 Å long, 20 Å wide tube, whereas the cytoplasmic vestibule approximates to a 20 Ð diameter sphere (giving rise to a long, 20 Å wide tube, whereas the cytoplasmic vestibule
approximates to a 20 Å diameter sphere (giving rise to a
circular outline in the figure). The only aqueous links
between the cytoplasmic vestibule and the approximates to a 20 Å diameter sphere (giving rise to a circular outline in the figure). The only aqueous links
between the cytoplasmic vestibule and the cell interior are
narrow (ϵ 8–9 Å wide) openings in the wall ly circular outline in the figure). The only aqueous links
between the cytoplasmic vestibule and the cell interior are
narrow $(< 8-9$ Å wide) openings in the wall lying directly
under the membrane surface (The cytoplasmic st between the cytoplasmic vestibule and the cell interior are
narrow ($\lt 8$ –9Å wide) openings in the wall lying directly
under the membrane surface. (The cytoplasmic structure
of the voltage-gated K^+ channel may have a narrow ($\lt 8$ –9 Å wide) openings in the wall lying directly
under the membrane surface. (The cytoplasmic structure
of the voltage-gated K⁺ channel may have a similar
design: Gulbis *et al* 2000) under the membrane surface
of the voltage-gated K⁺ c
design; Gulbis *et al.* 2000.)
Individual subunits make the voltage-gated K^+ channel may have a similar
sign; Gulbis *et al.* 2000.)
Individual subunits make tight contacts in the upper
rtion of the extracellular domain and within the

design; Gulbis et al. 2000.)
Individual subunits make tight contacts in the upper
portion of the extracellular domain and within the Individual subunits make tight contacts in the upper
portion of the extracellular domain and within the
membrane, and it will not be possible to determine
their exact boundaries in these regions until the portion of the extracellular domain and within the
membrane, and it will not be possible to determine
their exact boundaries in these regions until the
polynentide chains are completely traced However the membrane, and it will not be possible to determine
their exact boundaries in these regions until the
polypeptide chains are completely traced. However, the
two α -subunits can be identified unambiguously in their exact boundaries in these regions until the polypeptide chains are completely traced. However, the two α -subunits can be identified unambiguously, in polypeptide chains are completely traced. However, the
two α -subunits can be identified unambiguously, in
other regions, because of their clear structural similari-
ties. Thus it can be shown that the subunits have a two α -subunits can be identified unambiguously, in
other regions, because of their clear structural similari-
ties. Thus it can be shown that the subunits have a
small $(c\alpha, 10^{\circ})$ tilt tangential to the axis of the r other regions, because of their clear structural similarities. Thus it can be shown that the subunits have a small $(ca. 10^{\circ})$ tilt tangential to the axis of the receptor, giving the whole complex a slightly (right-handed ties. Thus it can be shown that the subunits have a small $(ca. 10^{\circ})$ tilt tangential to the axis of the receptor, giving the whole complex a slightly (right-handed) coiled configuration. small (ca. 10 $^{\circ}$) tilt tangential to the axis of the receptor, ving the whole complex a slightly (right-handed)
iled configuration.
The locations of the two α -subunits, α_{δ} and α_{γ} , are
dicated in the figure The δ -subunit lies anticlockwise of

coiled configuration.
The locations of the two α -subunits, α_{δ} and α_{γ} , are
indicated in the figure. The δ -subunit lies anticlockwise of
 α_{δ} next to the symmetry axis of the recentor dimer. The α_{δ} , next to the symmetry axis of the receptor dimer. The The locations of the two α -subunits, α_{δ} and α_{γ} , are dicated in the figure. The δ -subunit lies anticlockwise of , next to the symmetry axis of the receptor dimer. The specifies subunit separation the two indicated in the figure. The δ -subunit lies anticlockwise of α_{δ} , next to the symmetry axis of the receptor dimer. The single subunit separating the two α s resembles the α s more closely than the others, and single subunit separating the two α s resembles the α s
more closely than the others, and so is likely to be β , the
subunit having highest sequence homology to α (see also more closely than the others, and so is likely to be β , the subunit having highest sequence homology to α (see also Kubalek *et al.* 1987). However, the interpretation usually given favours α in this location (Ka subunit having highest sequence homology to Kubalek *et al.* 1987). However, the interpretation (Karlin 1993). The ACh-binding pockets are located about abalek *et al.* 1987). However, the interpretation usually
ven favours γ in this location (Karlin 1993).
The ACh-binding pockets are located about halfway
tween the extracellular ends of the α -subunits and the

given favours γ in this location (Karlin 1993).
The ACh-binding pockets are located about halfway
between the extracellular ends of the α -subunits and the
membrane (asterisks, figure 8); the gate of the closed The ACh-binding pockets are located about halfway
between the extracellular ends of the α -subunits and the
membrane (asterisks, figure 8); the gate of the closed
pore is near the middle of the membrane (upper arrow between the extracellular ends of the α -subunits and the membrane (asterisks, figure 8); the gate of the closed pore is near the middle of the membrane (upper arrow, figure 8): and the constriction part of the open por membrane (asterisks, figure 8); the gate of the closed
pore is near the middle of the membrane (upper arrow,
figure 8); and the constricting part of the open pore is
at the cytoplasmic membrane surface (lower arrow, pore is near the middle of the membrane (upper arrow, figure 8); and the constricting part of the open pore is at the cytoplasmic membrane surface (lower arrow, figure 8) figure 8); $\frac{3}{x}$
at the cy
figure 8).

9. THE VESTIBULES

Neurotransmitter-gated ion channels have evolved to S. THE VESTIBULES
Contain large vestibules, shaped by more than 70% of
the total protein mass. Much of this mass extending Neurotransmitter-gated ion channels have evolved to
contain large vestibules, shaped by more than 70% of
the total protein mass. Much of this mass extending
from the membrane may be involved in shaning the contain large vestibules, shaped by more than 70% of
the total protein mass. Much of this mass extending
from the membrane may be involved in shaping the *Phil. Trans. R. Soc. Lond.* B (2000) *Phil. Trans. R. Soc. Lond.* B (2000)

Fast synaptic transmission N. Unwin 1821
ACh-binding pockets and sites of attachment for regulatory molecules and other proteins (e.g. rapsyn) that are ACh-binding pockets and sites of attachment for regula-
tory molecules and other proteins (e.g. rapsyn) that are
concentrated at the synapse. However, an important
physiological function of these vestibules may also be to tory molecules and other proteins (e.g. rapsyn) that are
concentrated at the synapse. However, an important
physiological function of these vestibules may also be to
serve as preselectivity filters, making use of charged concentrated at the synapse. However, an important
physiological function of these vestibules may also be to
serve as preselectivity filters, making use of charged
groups at their mouths and on their inner walls to electro physiological function of these vestibules may also be to serve as preselectivity filters, making use of charged
groups at their mouths and on their inner walls to electro-
statically guide and concentrate the ions they select for
while screening out the ions they discriminate ag groups at their mouths and on their inner walls to electro-
statically guide and concentrate the ions they select for
while screening out the ions they discriminate against. The
ionic environment would consequently be modi statically guide and concentrate the ions they select for
while screening out the ions they discriminate against. The
ionic environment would consequently be modified close
to the narrow membrane-spanning pore, increasing while screening out the ions they discriminate against. The
ionic environment would consequently be modified close
to the narrow membrane-spanning pore, increasing the
efficiency of transport of the nermeant ions, and enha ionic environment would consequently be modified close
to the narrow membrane-spanning pore, increasing the
efficiency of transport of the permeant ions, and enhan-
cing the selectivity arising from their direct interactio to the narrow membrane-spanning pore, increasing the
efficiency of transport of the permeant ions, and enhan-
cing the selectivity arising from their direct interaction
with residues and/or backbone groups lining the const efficiency of transport of the permeant ions, and enhan-
cing the selectivity arising from their direct interaction
with residues and/or backbone groups lining the constric-
tion zone cing the selectivity arising from their direct interaction
with residues and/or backbone groups lining the constric-
tion zone.

Although we will need to see the whole receptor in tion zone.
Although we will need to see the whole receptor in
atomic detail before we know precisely the strategic
locations of the charged groups influencing its cation Although we will need to see the whole receptor in
atomic detail before we know precisely the strategic
locations of the charged groups influencing its cation
selectivity some features of the cytoplasmic vestibule locations of the charged groups influencing its cation
selectivity, some features of the cytoplasmic vestibule locations of the charged groups influencing its cation
selectivity, some features of the cytoplasmic vestibule
already apparent highlight the probable importance of
the preselection process. The parrow openings into this selectivity, some features of the cytoplasmic vestibule
already apparent highlight the probable importance of
the preselection process. The narrow openings into this
vestibule are made between $ca = 30 \text{ Å}$ long α -belic already apparent highlight the probable importance of
the preselection process. The narrow openings into this
vestibule are made between ca . 30\AA long α -helices
extending down from the base of each subunit and the preselection process. The narrow openings into this
vestibule are made between $ca. 30 \text{\AA}$ long α -helices
extending down from the base of each subunit and
coming together on the central axis of the recentor vestibule are made between ca . 30\AA long α -helices extending down from the base of each subunit and coming together on the central axis of the receptor, forming an inverted nentagonal cone. Clearly these extending down from the base of each subunit and
coming together on the central axis of the receptor,
forming an inverted pentagonal cone. Clearly these coming together on the central axis of the receptor,
forming an inverted pentagonal cone. Clearly these
openings would serve as molecular sieves, preventing
impermeant cytoplasmic molecules from reaching the forming an inverted pentagonal cone. Clearly these
openings would serve as molecular sieves, preventing
impermeant cytoplasmic molecules from reaching the
vicinity of the pore. However, a stretch of the polyimpermeant cytoplasmic molecules from reaching the vicinity of the pore. However, a stretch of the polyimpermeant cytoplasmic molecules from reaching the
vicinity of the pore. However, a stretch of the poly-
peptide chains between M3 and M4, containing heptad
reneats of negatively charged residues, can be identified vicinity of the pore. However, a stretch of the poly-
peptide chains between M3 and M4, containing heptad
repeats of negatively charged residues, can be identified
with the 30 Å long α -helices (Miyazawa et al. 1999) an peptide chains between M3 and M4, containing heptad
repeats of negatively charged residues, can be identified
with the 30 Å long α-helices (Miyazawa *et al.* 1999), and
traced tentatively through the 3D densities. The repeats of negatively charged residues, can be identified with the 30\AA long α -helices (Miyazawa *et al.* 1999), and traced tentatively through the $3D$ densities. The with the 30 Å long α -helices (Miyazawa *et al.* 1999), and traced tentatively through the 3D densities. The repeating residues are found to line the sides of the openings: moreover this stretch of amino acids contains traced tentatively through the 3D densities. The
repeating residues are found to line the sides of the
openings; moreover, this stretch of amino acids contains
additional negatively charged residues which line the repeating residues are found to line the sides of the openings; moreover, this stretch of amino acids contains additional negatively charged residues which line the vestibule's inner wall. Passing ions must be strongly openings; moreover, this stretch of amino acids contains additional negatively charged residues which line the vestibule's inner wall. Passing ions must be strongly influenced by these charges, especially at the openings, vestibule's inner wall. Passing ions must be strongly
influenced by these charges, especially at the openings,
the widest dimensions of which do not exceed signifi-
cantly the diameter of an ion including its first hydrati influenced by these charges, especially at the openings,
the widest dimensions of which do not exceed signifi-
cantly the diameter of an ion including its first hydration
shell. On this side of the membrane, therefore, the the widest dimensions of which do not exceed significantly the diameter of an ion including its first hydration shell. On this side of the membrane, therefore, the receptor creates a cation-stabilizing environment and cantly the diameter of an ion including its first hydration
shell. On this side of the membrane, therefore, the
receptor creates a cation-stabilizing environment, and
seems designed largely to exclude anions from the vicin shell. On this side of the membrane, therefore, the receptor creates a cation-stabilizing environment, and seems designed largely to exclude anions from the vicinity of the nore receptor creates a cation-stabilizing environment, and
seems designed largely to exclude anions from the vicinity
of the pore.

The extracellular vestibule is architecturally distinct of the pore.
The extracellular vestibule is architecturally distinct
from the cytoplasmic vestibule, yet presumably it partici-
pates similarly as a preselectivity filter, since electro-The extracellular vestibule is architecturally distinct
from the cytoplasmic vestibule, yet presumably it partici-
pates similarly as a preselectivity filter, since electro-
physiological experiments have shown that there from the cytoplasmic vestibule, yet presumably it partici-
pates similarly as a preselectivity filter, since electro-
physiological experiments have shown that there is no
marked preference for cations to go in one directi pates similarly as a preselectivity filter, since electro-
physiological experiments have shown that there is no
marked preference for cations to go in one direction
across the membrane. It is notable that the cylindrical physiological experiments have shown that there is no
marked preference for cations to go in one direction
across the membrane. It is notable that the cylindrical
shape and ca 10 \AA radius would provide a route that is marked preference for cations to go in one direction
across the membrane. It is notable that the cylindrical
shape and *ca*. 10 Å radius would provide a route that is
narrow enough for the charged groups on the inner wall across the membrane. It is notable that the cylindrical shape and ca . 10 Å radius would provide a route that is narrow enough for the charged groups on the inner wall to influence ions at the centre but not too narrow to shape and ca . 10 Å radius would provide a route that is narrow enough for the charged groups on the inner wall
to influence ions at the centre but not too narrow to
restrict their diffusion. The average net negative char narrow enough for the charged groups on the inner wall to influence ions at the centre but not too narrow to restrict their diffusion. The average net negative charge per subunit in the extracellular part of the receptor, estimated from the amino-acid sequences is $-11e$ (Unw restrict their diffusion. The average net negative charge
per subunit in the extracellular part of the receptor,
estimated from the amino-acid sequences, is $-11e$ (Unwin
1989) This figure is the same as that for the fastper subunit in the extracellular part of the receptor,
estimated from the amino-acid sequences, is $-11e$ (Unwin
1989). This figure is the same as that for the fast-acting
enzyme acetylcholinesterase from Tarbeda (Nolte e estimated from the amino-acid sequences, is $-11e$ (Unwin 1989). This figure is the same as that for the fast-acting enzyme, acetylcholinesterase from *Torpedo* (Nolte *et al.* 1980; Ripoll *et al.* 1993), where the whole 1989). This figure is the same as that for the fast-acting enzyme, acetylcholinesterase from *Torpedo* (Nolte *et al.* 1980; Ripoll *et al.* 1993), where the whole protein surface plays a role in producing an electrostatic field that guides the positively charged ACb substrate to 1980; Ripoll *et al.* 1993), where the whole protein surface plays a role in producing an electrostatic field that guides the positively charged ACh substrate to the active site. It is easy to imagine that the receptor us the positively charged ACh substrate to the active site. It is easy to imagine that the receptor uses its predominantly the positively charged ACh substrate to the active site. It
is easy to imagine that the receptor uses its predominantly
negatively charged surface to guide the ACh molecules
and the inorganic cations in a similar fashion (is easy to imagine that the receptor uses its predominantly
negatively charged surface to guide the ACh molecules
and the inorganic cations in a similar fashion (for
example see Adcock et al. 1998) negatively charged surface to guand the inorganic cations in example, see Adcock *et al.* 1998).

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Figure 9. The higher resolution maps suggest that the gate
may be made by leucine and valine side-chains creating a Figure 9. The higher resolution maps suggest that the gate
may be made by leucine and valine side-chains creating a
hydrophobic girdle around the pore. In the absence of polar Figure 9. The higher resolution maps suggest that the gate
may be made by leucine and valine side-chains creating a
hydrophobic girdle around the pore. In the absence of polar
groups to provide electrostatic stabilization, may be made by leucine and valine side-chains creating a
hydrophobic girdle around the pore. In the absence of polar
groups to provide electrostatic stabilization, the ion would
retain its hydration shell and then be too l hydrophobic girdle around the pore. In the absence of polar
groups to provide electrostatic stabilization, the ion would
retain its hydration shell and then be too large to pass through
the central hole groups to provide
retain its hydratio
the central hole.

10. THE GATE

10. THE GATE
The gate of the channel lies at the centre of the ring of
the best M ? α -belical segments where they come closest The gate of the channel lies at the centre of the ring of
five bent M2 α -helical segments where they come closest
to the axis of the membrane-spanning pore. In the 4.6 \AA The gate of the channel lies at the centre of the ring of
five bent $M2 \alpha$ -helical segments where they come closest
to the axis of the membrane-spanning pore. In the 4.6 Å
resolution man of the closed-channel form of the five bent M2 α -helical segments where they come closest
to the axis of the membrane-spanning pore. In the 4.6 Å
resolution map of the closed-channel form of the receptor,
it is seen as a narrow strip of density—no more to the axis of the membrane-spanning pore. In the 4.6\AA resolution map of the closed-channel form of the receptor, it is seen as a narrow strip of density—no more than two rings of side-chains thick—bridging the pore resolution map of the closed-channel form of the receptor,
it is seen as a narrow strip of density—no more than two
rings of side-chains thick—bridging the pore (Miyazawa *et al.* 1999). The level of the bridging density is near the rings of side-chains thick—bridging the pore (Miyazawa *et al.* 1999). The level of the bridging density is near the middle of the membrane (Unwin 1993), where the dielectric barrier to ion permeation is greatest (Parsegi *et al.* 1999). The level of the bridging density is near the middle of the membrane (Unwin 1993), where the dielectric barrier to ion permeation is greatest (Parsegian 1969; Roux & MacKinnon 1999). middle of the membrane (Un
tric barrier to ion permeation
Roux & MacKinnon 1999).
The precise pature of the tric barrier to ion permeation is greatest (Parsegian 1969;
Roux & MacKinnon 1999).
The precise nature of the gate—whether it forms a

Roux & MacKinnon 1999).
The precise nature of the gate—whether it forms a the physical occlusion, or is better described as a zone of low
polarizability that reinforces the dielectric barrier to jou The precise nature of the gate—whether it forms a
physical occlusion, or is better described as a zone of low
polarizability that reinforces the dielectric barrier to ion
permeation—has not yet been clarified Farlier studi polarizability that reinforces the dielectric barrier to ion
permeation—has not yet been clarified. Earlier studies (figure 4) had emphasized the likely importance of the permeation—has not yet been clarified. Earlier studies
(figure 4) had emphasized the likely importance of the
ring of conserved leucine residues (α Leu251), and had
suggested they might project inwards from the M² (figure 4) had emphasized the likely importance of the
ring of conserved leucine residues $(\alpha$ Leu251), and had
suggested they might project inwards from the M2
segments associating side-to-side to form an occluding ring of conserved leucine residues (α Leu251), and had suggested they might project inwards from the M2 segments, associating side-to-side to form an occluding gate But the side-chain densities in the higher resolution suggested they might project inwards from the M2
segments, associating side-to-side to form an occluding
gate. But the side-chain densities in the higher resolution
maps do not come close enough to the pore axis to segments, associating side-to-side to form an occluding
gate. But the side-chain densities in the higher resolution
maps do not come close enough to the pore axis to
account for such a simple arrangement gate. But the side-chain densities in the l
maps do not come close enough to the
account for such a simple arrangement.
Apparently there is a hole of ca $3.5\,\text{\AA}$ aps do not come close enough to the pore axis to
count for such a simple arrangement.
Apparently there is a hole of *ca*. 3.5 Å radius along the
ntral axis of the pore where the gate is located. This is

account for such a simple arrangement.

Apparently there is a hole of *ca*. 3.5 Å radius along the

central axis of the pore where the gate is located. This is

only slightly smaller than the radius of a Na⁺ or K⁺ ion Apparently there is a hole of *ca*. 3.5 Å radius along the central axis of the pore where the gate is located. This is only slightly smaller than the radius of a Na⁺ or K⁺ ion having a single hydration shell. Therefor only slightly smaller than the radius of a Na $^+$ or K $^+$ ion Central axis of the pore where the gate is located. This is
only slightly smaller than the radius of a Na^+ or K^+ ion
| having a single hydration shell. Therefore, to be effective in preventing ion permeation, the gate should counter
any opportunity for electrostatic stabilization by polar having a single hydration shell. Therefore, to be effective
in preventing ion permeation, the gate should counter
any opportunity for electrostatic stabilization by polar
groups that could liberate the ions from their hydr in preventing ion permeation, the gate should counter
any opportunity for electrostatic stabilization by polar
groups that could liberate the ions from their hydration
shells. It needs to form a completely hydrophobic gird any opportunity for electrostatic stabilization by polar
groups that could liberate the ions from their hydration
shells. It needs to form a completely hydrophobic girdle
around the hole Two rings of hydrophobic residues c groups that could liberate the ions from their hydration
shells. It needs to form a completely hydrophobic girdle
around the hole. Two rings of hydrophobic residues could
be part of this girdle: one including the conserved shells. It needs to form a completely hydrophobic girdle around the hole. Two rings of hydrophobic residues could
be part of this girdle: one including the conserved leucine around the hole. Two rings of hydrophobic residues could
be part of this girdle: one including the conserved leucine
and the other including a valine $(\alpha\text{Val}255; \text{ also highly}$
conserved in ACb recentors) one turn of a helix awa be part of this girdle: one including the conserved leucine
and the other including a valine $(\alpha \text{Val}255; \text{ also highly}$
conserved in ACh receptors), one turn of a helix away.
Residues in both these rings can be labelled by small and the other including a valine $(\alpha \text{Val} 255;$ also highly conserved in ACh receptors), one turn of a helix away.
Residues in both these rings can be labelled by small **Residues in both these rings can be labelled by small**
Phil. Trans. R. Soc. Lond. B (2000)

uncharged photoactivable compounds when the channel
is closed (White & Cohen 1992 Blanton et al. 1998) uncharged photoactivable compounds when the cha
is closed (White & Cohen 1992; Blanton *et al.* 1998).
Figure 9 shows how the leucines may interact side charged photoactivable compounds when the channel
closed (White & Cohen 1992; Blanton *et al.* 1998).
Figure 9 shows how the leucines may interact side-to-
le with alanine and/or serine residues on neighbouring

is closed (White & Cohen 1992; Blanton *et al.* 1998).
Figure 9 shows how the leucines may interact side-to-
side with alanine and/or serine residues on neighbouring
M₂ segments, rather than with each other, to form the Figure 9 shows how the leucines may interact side-to-
side with alanine and/or serine residues on neighbouring
 $M2$ segments, rather than with each other, to form the
hydrophobic girdle. Since the $M2$ segments are not side with alanine and/or serine residues on neighbouring
M2 segments, rather than with each other, to form the
hydrophobic girdle. Since the M2 segments are not
closely packed and draw away from the pore axis and $M2$ segments, rather than with each other, to form the hydrophobic girdle. Since the $M2$ segments are not closely packed and draw away from the pore axis and hence from each other on either side of the bend this hydrophobic girdle. Since the M2 segments are not closely packed and draw away from the pore axis and hence from each other on either side of the bend, this may well be the only set of interactions between these closely packed and draw away from the pore axis and
hence from each other on either side of the bend, this
may well be the only set of interactions between these
segments. The resulting limited stability of the structure hence from each other on either side of the bend, this
may well be the only set of interactions between these
segments. The resulting limited stability of the structure
would ensure its easy disruption and reassembly by may well be the only set of interactions between these
segments. The resulting limited stability of the structure
would ensure its easy disruption and reassembly by
conformational changes initiated at the binding sites segments. The resulting limited stability of the struct
would ensure its easy disruption and reassembly
conformational changes initiated at the binding sites.
Other pentameric structures composed of bundles of

would ensure its easy disruption and reassembly by
conformational changes initiated at the binding sites.
Other pentameric structures composed of bundles of α -
helices, such as phospholamban (Adams *et al.* 1995), the
 Other pentameric structures composed of bundles of α -helices, such as phospholamban (Adams *et al.* 1995), the matrix protein COMP (Malashkevich *et al.* 1996) and the mechanosensitive channel (Chang *et al.* 1998) all helices, such as phospholamban (Adams *et al.* 1995), the matrix protein COMP (Malashkevich *et al.* 1996) and the mechanosensitive channel (Chang *et al.* 1998), all contain rings of hydrophobic side-chains lying closer t matrix protein COMP (Malashkevich *et al.* 1996) and the mechanosensitive channel (Chang *et al.* 1998), all contain rings of hydrophobic side-chains lying closer to the pore mechanosensitive channel (Chang *et al.* 1998), all contain
rings of hydrophobic side-chains lying closer to the pore
axis than those of the receptor. These side-chains could
function as occluding gates. However, the α rings of hydrophobic side-chains lying closer to the pore axis than those of the receptor. These side-chains could
function as occluding gates. However, the α -helical
bundle structures involve more extensive side-to-si axis than those of the receptor. These side-chains could
function as occluding gates. However, the α -helical
bundle structures involve more extensive side-to-side
interactions increasing their stability and making them function as occluding gates. However, the α -helical
bundle structures involve more extensive side-to-side
interactions, increasing their stability and making them
less suited for rapid conversion between two widely bundle structures involve more extensive side-to-side
interactions, increasing their stability and making them
less suited for rapid conversion between two widely
distinct and precisely defined states interactions, increasing their stability
less suited for rapid conversion b
distinct and precisely defined states.
The proposed hydrophobic girdle Its less suited for rapid conversion between two widely
distinct and precisely defined states.
The proposed hydrophobic girdle of the ACh receptor

contrasts with the central region of the K^+ channel, The proposed hydrophobic girdle of the ACh receptor contrasts with the central region of the K^+ channel, where there is a large $(ca. 5 \text{ Å radius})$ water-filled cavity and helix dipoles positioned so as to overcome electrocontrasts with the central region of the K^+ channel,
where there is a large (*ca*. 5 Å radius) water-filled cavity
and helix dipoles positioned so as to overcome electro-
static destabilization of the ion (Doyle *et al* where there is a large $\langle a, 5 \text{ Å} \rangle$ radius) water-filled cavity
and helix dipoles positioned so as to overcome electro-
static destabilization of the ion (Doyle *et al.* 1998).
However the M₂ segments move ca $\frac{2 \$ and helix dipoles positioned so as to overcome electro-
static destabilization of the ion (Doyle *et al.* 1998).
However, the M2 segments move *ca.* 2 Å away from the
pore axis in the middle of the membrane when the static destabilization of the ion (Doyle *et al.* 1998).
However, the M2 segments move *ca.* 2 Å away from the pore axis in the middle of the membrane when the channel opens (figure 5). The two central regions would pore axis in the middle of the membrane when the channel opens (figure 5). The two central regions would pore axis in the middle of the membrane when the channel opens (figure 5). The two central regions would therefore have similar dimensions if the comparison were made with the receptor in the open-channel form channel opens (figure 5). The two central regions
therefore have similar dimensions if the comparisor
made with the receptor in the open-channel form. made with the receptor in the open-channel form.
11. ACh-BINDING POCKETS

The cavities making the ACh-binding pockets lie within the α -subunits about 45 Å away from the gate. At The cavities making the ACh-binding pockets lie
within the α -subunits about 45 Å away from the gate. At
4.6 Å resolution, narrow tunnels can be seen connecting
the cavities to the extracellular vestibule (forms 10 within the α -subunits about 45 Å away from the gate. At 4.6 Å resolution, narrow tunnels can be seen connecting
the cavities to the extracellular vestibule (figure 10;
Mivazawa et al. 1999) These tunnels, which are 10– 4.6 Å resolution, narrow tunnels can be seen connecting
the cavities to the extracellular vestibule (figure 10;
Miyazawa *et al.* 1999). These tunnels, which are 10–15 Å
long are shaned largely by twisted B-sheet strands, the cavities to the extracellular vestibule (figure 10; Miyazawa *et al.* 1999). These tunnels, which are $10-15 \text{ Å}$ long, are shaped largely by twisted β -sheet strands, and Miyazawa *et al.* 1999). These tunnels, which are $10-15$ Å long, are shaped largely by twisted β -sheet strands, and open into the upper (furthest from the membrane) ends of the cavities. The tunnels very likely repres long, are shaped largely by twisted β -sheet strands, and
open into the upper (furthest from the membrane) ends
of the cavities. The tunnels very likely represent the
primary entry routes for ACb into the binding pocket open into the upper (furthest from the membrane) ends
of the cavities. The tunnels very likely represent the
primary entry routes for ACh into the binding pockets,
first because the electrostatic field would favour its mov of the cavities. The tunnels very likely represent the primary entry routes for ACh into the binding pockets, first because the electrostatic field would favour its movement, along with the inorganic cations, into the vest primary entry routes for ACh into the binding pockets, and second because the tunnel-cavity design parallels the ACh-binding region of acetylcholinesterase, consisting of and second because the tunnel-cavity design parallels the
ACh-binding region of acetylcholinesterase, consisting of
a narrow 'gorge' about 20 Å long widening out into the
active site at its base (Sussman et al. 1991). The ACh-binding region of acetylcholinesterase, consisting of
a narrow 'gorge' about 20 Å long widening out into the
active site at its base (Sussman *et al.* 1991). The gorge
facilitates the ranid transfer of ACh into the act a narrow 'gorge' about 20 Å long widening out into the active site at its base (Sussman *et al.* 1991). The gorge facilitates the rapid transfer of ACh into the active site, and the tunnels of the receptor would presumabl active site at its base (Sussman *et al.* 1991). The gorge facilitates the rapid transfer of ACh into the active site, and the tunnels of the receptor would presumably function in an analogous way. facilitates the rapid transfer of
and the tunnels of the rec
function in an analogous way.
Although the cavities them d the tunnels of the receptor would presumably

nction in an analogous way.

Although the cavities themselves lie entirely within
 ϵ , γ -subunits (see below) side-chains from neigh-

function in an analogous way.
Although the cavities themselves lie entirely within
the α -subunits (see below), side-chains from neigh-
houring δ - and γ -subunits may well make up part of the Although the cavities themselves lie entirely within
the α -subunits (see below), side-chains from neigh-
bouring δ - and γ -subunits may well make up part of the
structure shaning the tunnels and other regions of t the α -subunits (see below), side-chains from neighbouring δ - and γ -subunits may well make up part of the structure shaping the tunnels and other regions of the vestibule in the vicinity of the cavities. Non α bouring δ - and γ -subunits may well make up part of the structure shaping the tunnels and other regions of the vestibule in the vicinity of the cavities. Non α -subunits are indeed likely to participate in the proc structure shaping the tunnels and other regions of the vestibule in the vicinity of the cavities. Non α -subunits are indeed likely to participate in the process by which ACh is selectively guided into the binding pocket by contri-
buting charged groups in strategic locatio indeed likely to participate in the process by which ACh
is selectively guided into the binding pocket by contri-
buting charged groups in strategic locations or aromatic
side-chains that interact selectively with the quat is selectively guided into the binding pocket by contributing charged groups in strategic locations or aromatic
side-chains that interact selectively with the quaternary

Figure 10. Detail at 4.6 Å resolution showing tunnels at the top of the cavities forming the ACh-binding pockets. (*a*) α_{δ} contains an 'entry' tunnel (red arrow) connecting to the water-filled vestibule (bottom): (Figure 10. Detail at 4.6 Å resolution showing tunnels at the top of the cavities forming the ACh-binding pockets. (*a*) α_{δ} contains an 'entry' tunnel (red arrow) connecting to the water-filled vestibule (bottom); (Figure 10. Detail at 4.6 Å resolution showing tunnels at the top of the cavities forming the ACh-binding pockets. (*a*) α_{δ} contains an 'entry' tunnel (red arrow) connecting to the water-filled vestibule (bottom); ($\overline{ }$ an 'entry' tunnel (red arrow) connecting to the water-filled vestibule (bottom); (*b*) α_{γ} contains another tunnel (horizontal red arrow) in addition to the entry tunnel, which may serve as a route for release of ACh $\mathbf S$

Figure 11. α -carbon tracing of a polypeptide chain containing
a pair of adjacent cysteine residues cross-linked to each other
in the alternative right-handed (broken line) and left-handed Figure 11. α -carbon tracing of a polypeptide chain containing
a pair of adjacent cysteine residues cross-linked to each other
in the alternative right-handed (broken line) and left-handed
(full line) conformations. The a pair of adjacent cysteine residues cross-linked to each other
in the alternative right-handed (broken line) and left-handed
(full line) conformations. The two conformations make different bends in the polypeptide chain. (The torsion angles, ω , of the included *cis* peptide bond are indicated.)

ammonium group (Ripoll *et al.* 1993). These interactions,
next to the site where ACb finally resides may account ammonium group (Ripoll *et al.* 1993). These interactions, next to the site where ACh finally resides, may account for some of the chemical labelling and site-directed mutaammonium group (Ripoll *et al.* 1993). These interactions, next to the site where ACh finally resides, may account for some of the chemical labelling and site-directed muta-genesis results interpreted to indicate that the next to the site where ACh finally resides, may account
for some of the chemical labelling and site-directed muta-
genesis results interpreted to indicate that the binding
sites lie at the $\alpha-\alpha$ and $\alpha-\delta$ interfaces (Cz for some of the chemical labelling and site-directed muta-
genesis results interpreted to indicate that the binding
sites lie at the $\alpha-\gamma$ and $\alpha-\delta$ interfaces (Czajkowski & genesis results interpreted to indicate that the binding
sites lie at the $\alpha-\gamma$ and $\alpha-\delta$ interfaces (Czajkowski &
Karlin 1995; Sine *et al.* 1995; Chiara & Cohen 1997; Chan-
geux & Edelstein 1998) sites lie at the $\alpha-\gamma$ and
Karlin 1995; Sine *et al.* 199
geux & Edelstein 1998).
In addition to the 'ent Exercise 1995; Sine *et al.* 1995; Chiara & Cohen 1997; Chan-
ux & Edelstein 1998).
In addition to the 'entry' tunnel, α_{γ} contains another
nnel, at a level slightly closer to the membrane

geux & Edelstein 1998).
In addition to the 'entry' tunnel, α_{γ} contains another
tunnel, at a level slightly closer to the membrane,
connecting the binding pocket to the external surround-In addition to the 'entry' tunnel, α_{γ} contains another
tunnel, at a level slightly closer to the membrane,
connecting the binding pocket to the external surround-
ing (dotted line figure 3: figure 10b) This may serv tunnel, at a level slightly closer to the membrane, connecting the binding pocket to the external surroundings (dotted line, figure 3; figure 10*b*). This may serve as an alternative route through which the ACh can be ings (dotted line, figure 3; figure 10*b*). This may serve as
an alternative route through which the ACh can be
released. Although α_{δ} does not contain an equivalent
tunnel in the absence of ACh (figure 10*a*) both s an alternative route through which the ACh can be released. Although α_{δ} does not contain an equivalent tunnel in the absence of ACh (figure 10*a*), both subunits adopt a conformation recembling that of α when ACh released. Although α_{δ} does not contain an equivalent
tunnel in the absence of ACh (figure 10*a*), both subunits
adopt a conformation resembling that of α_{γ} when ACh is
present (+ACh figure 3) An 'evit' tunnel m tunnel in the absence of ACh (figure 10*a*), both subunits
adopt a conformation resembling that of α_{γ} when ACh is
present (+ACh, figure 3). An 'exit' tunnel may therefore appear in α_{δ} once ACh has bound.

appear in α_{δ} once ACh has bound.
The different conformations of α_{δ} and α_{γ} around
the cavities, before activation, were apparent from the
non-equivalent orientations of the three surrounding rods The different conformations of α_{δ} and α_{γ} around
the cavities, before activation, were apparent from the
non-equivalent orientations of the three surrounding rods
of density ($-\Delta C$ b figure 3) At bigher resoluti the cavities, before activation, were apparent from the non-equivalent orientations of the three surrounding rods of density $(-ACh,$ figure 3). At higher resolution, it

Extracting the differences between α_{δ} before (grey)
pear in α_{δ} once ACh has bound.
The different conformations of α_{δ} and α_{γ} around
e cavities, before activation, were apparent from the linked to M2 activation. Tentative α -carbon tracings are shown of the α_s - and α_s -chains (*Torpedo* α 166-211) around an ACh activation. Tentative α -carbon tracings are shown of the α_{δ} - and α_{γ} -chains (*Torpedo* α 166-211) around an ACh
molecule in the binding pocket; differences between the two
tracings resemble the differences α_{δ} - and α_{γ} -chains (*Torpedo* α 166-211) around an ACh
molecule in the binding pocket; differences between the two
tracings resemble the differences between α_{δ} before (grey)
and after (nink) activation molecule in the binding pocket; differences between the two
tracings resemble the differences between α_{δ} before (grey)
and after (pink) activation (see figure 3). The comparison
suggests that ACb binding might induc suggests that ACh-binding might induce displacements near and after (pink) activation (see figure 3). The comparison
suggests that ACh-binding might induce displacements near
the pair of cysteine residues (arrow), which in turn might be
linked to M2, drawing the gate-forming leuc suggests that ACh-binding might induce displacements near
the pair of cysteine residues (arrow), which in turn might be
linked to M2, drawing the gate-forming leucine side-chain
away from the axis of the pare. The view is the pair of cysteine residues (arrow), which in turn might be
linked to M2, drawing the gate-forming leucine side-chain
away from the axis of the pore. The view is normal to the
plane of the membrane linked to $M2$, drawing the away from the axis of the plane of the membrane.

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appears that α_{δ} creates a more symmetrical and 'tighter' cavity (figure 10*a*) than the one in α _v (figure 10*b*), because appears that α_{δ} creates a more symmetrical and 'tighter'
cavity (figure 10*a*) than the one in α_{γ} (figure 10*b*), because
the α_{δ} -subunit in this region is 'pulled' towards the δ -
subunit (vellow arrow cavity (figure 10*a*) than the one in α_{γ} (figure 10*b*), because
the α_{δ} -subunit in this region is 'pulled' towards the δ -
subunit (yellow arrow, figure 10*a*). The (conical) shapes of
the two cavities are q the α_{δ} -subunit in this region is 'pulled' towards the δ -
subunit (yellow arrow, figure 10*a*). The (conical) shapes of
the two cavities are quite similar and complement well
the Van der Waal's surfaces of the ACh subunit (yellow arrow, figure 10a). The (conical) shapes of the two cavities are quite similar and complement well the Van der Waal's surfaces of the ACh molecule when it the two cavities are quite similar and complement well
the Van der Waal's surfaces of the ACh molecule when it
is seated slightly tilted with the quaternary ammonium
 K
group uppermost (figure 10c) In this orientation th the Van der Waal's surfaces of the ACh molecule when it
is seated slightly tilted with the quaternary ammonium
group uppermost (figure 10*c*). In this orientation, the
angle formed by the quaternary ammonium group and is seated slightly tilted with the quaternary ammonium
group uppermost (figure 10 c). In this orientation, the
angle formed by the quaternary ammonium group and
the membrane normal is about 45° in agreement with group uppermost (figure 10 c). In this orientation, the angle formed by the quaternary ammonium group and the membrane normal is about 45° , in agreement with the result obtained by solid-state nuclear magnetic resoangle formed by the quaternary ammonium group and
the membrane normal is about 45° , in agreement with
the result obtained by solid-state nuclear magnetic reso-
nance (Williamson *et al.* 2000) the membrane normal is about
the result obtained by solid-state
nance (Williamson *et al.* 2000).
The stretch of polynentide im the result obtained by solid-state nuclear magnetic resonance (Williamson *et al.* 2000).
The stretch of polypeptide immediately preceding M1

the densities composing the two α -subunits, and shown to be involved in shaping the cavity wall. Thus the cavity-(Torpedo α 166–211) can now be traced tentatively though
the densities composing the two α -subunits, and shown to
be involved in shaping the cavity wall. Thus the cavity-
lining rod furthest from the recentor axis (f the densities composing the two α -subunits, and shown to
be involved in shaping the cavity wall. Thus the cavity-
lining rod furthest from the receptor axis (figure 3) is an
 α -helix that extends down to the membrane be involved in shaping the cavity wall. Thus the cavity-
lining rod furthest from the receptor axis (figure 3) is an
 α -helix that extends down to the membrane surface.
Turl98 which can be photolabelled by the agonist lining rod furthest from the receptor axis (figure 3) is an α -helix that extends down to the membrane surface.
Tyr198, which can be photolabelled by the agonist
nicotine (Middleton & Coben 1991) is at the upper end of x-helix that extends down to the membrane surface.
Tyr198, which can be photolabelled by the agonist
nicotine (Middleton & Cohen 1991), is at the upper end of
the helix and faces the cavity This whole stretch of noly-Tyr198, which can be photolabelled by the agonist
nicotine (Middleton & Cohen 1991), is at the upper end of
the helix and faces the cavity. This whole stretch of poly-
pentide chain encircles the cavity and contains a β nicotine (Middleton & Cohen 1991), is at the upper end of
the helix and faces the cavity. This whole stretch of poly-
peptide chain encircles the cavity, and contains a β -sheet
haimin opposite the α -helix which draw the helix and faces the cavity. This whole stretch of poly-
peptide chain encircles the cavity, and contains a β -sheet
hairpin opposite the α -helix, which draws away from the
helix in the unner portion defining the peptide chain encircles the cavity, and contains a β -sheet hairpin opposite the α -helix, which draws away from the ohelix in the upper portion, defining the cavity space.

12. A HYPOTHETICAL SWITCH CONTROLLING ACTIVATION

EXAMPOTHETICAL SWITCH CONTROLLING

Biophysical evidence has shown that ACh receptor

haves as an allosteric protein undergoing concerted 'all Biophysical evidence has shown that ACh receptor
behaves as an allosteric protein, undergoing concerted 'all
or none' transitions consistent with the Monod–Wyman– Biophysical evidence has shown that ACh receptor
behaves as an allosteric protein, undergoing concerted 'all
or none' transitions consistent with the Monod–Wyman–
Changeux model (Monod *et al.* 1965). For example, the
chan or none' transitions consistent with the Monod–Wyman–Changeux model (Monod *et al.* 1965). For example, the channels occasionally open spontaneously, and do so with the same conductance properties as the ACh-activated Changeux model (Monod *et al.* 1965). For example, the channels occasionally open spontaneously, and do so with the same conductance properties as the ACh-activated channels (Jackson 1994). It appears that the protein int channels occasionally open spontaneously, and do so with
the same conductance properties as the ACh-activated
channels (Jackson 1994). It appears that the protein inter-
converts between two discrete 'pre-existing' closedthe same conductance properties as the ACh-activated the control of gating (Grosman *et al.* 2000). It is plausible channels (Jackson 1994). It appears that the protein inter-
converts between two discrete 'pre-existing' c open-channel forms, and that ACh binding simply alters the equilibrium in favour of the open-channel form. The open-channel forms, and that ACh binding simply alters
the equilibrium in favour of the open-channel form. The
alternative forms of an allosteric protein are often
described in terms of a tense or T state, where a subunit the equilibrium in favour of the open-channel form. The alternative forms of an allosteric protein are often described in terms of a tense, or T state, where a subunit is constrained to resist the change needed for ligand alternative forms of an allosteric protein are often
described in terms of a tense, or T state, where a subunit is
constrained to resist the change needed for ligand
binding and a relaxed or R state, where these described in terms of a tense, or T state, where a subunit is
constrained to resist the change needed for ligand
binding, and a relaxed, or R state, where these
constraints are relieved. This description also readily constrained to resist the change needed for ligand
binding, and a relaxed, or R state, where these
constraints are relieved. This description also readily
applies to the receptor. The structures shaping the two binding, and a relaxed, or R state, where these
constraints are relieved. This description also readily
applies to the receptor. The structures shaping the two
hinding pockets in the α -subunits are not equivalent constraints are relieved. This description also readily applies to the receptor. The structures shaping the two binding pockets in the α -subunits are not equivalent applies to the receptor. The structures shaping the two
binding pockets in the α -subunits are not equivalent
initially (despite their compositions being the same)
because of the unequal constraints imposed by interbinding pockets in the α -subunits are not equivalent
initially (despite their compositions being the same)
because of the unequal constraints imposed by inter-
actions with different neighbouring subunits. So, the initially (despite their compositions being the same)
because of the unequal constraints imposed by inter-
actions with different neighbouring subunits. So the
receptor is initially in the T state However, the two struc- \Box because of the unequal constraints imposed by inter-
 \Box actions with different neighbouring subunits. So the
 \Box receptor is initially in the T state. However, the two struc- U tures become more similar upon activation, indicating receptor is initially in the T state. However, the two structures become more similar upon activation, indicating
that the binding of ACh (partially) overcomes these
constraints. The receptor therefore changes into a less tures become more similar upon activation, indicating
that the binding of ACh (partially) overcomes these
constraints. The receptor therefore changes into a less
distorted conformation which is closer to the R state that the binding of ACh (partially) overcomes the constraints. The receptor therefore changes into a 1 distorted conformation, which is closer to the R state. The α -binding region is the one changed most by the state.

nstraints. The receptor therefore changes into a less
storted conformation, which is closer to the R state.
The α_{δ} -binding region is the one changed most by the
osteric transition, and ends un resembling the binding distorted conformation, which is closer to the R state.
The α_{δ} -binding region is the one changed most by the allosteric transition, and ends up resembling the binding region of α_{γ} in the initial state (see figure 3). How does \perp ACh convert α_{δ} into the activated conformation resem-In the initial state (see figure 3). How does α into the activated conformation resemallosteric transition, and ends up resembling the binding
region of α_{γ} in the initial state (see figure 3). How does
ACh convert α_{δ} into the activated conformation resem-
bling α ? The structural results po region of α_{γ} in the initial state (see figure 3). How does
ACh convert α_{δ} into the activated conformation resem-
bling α_{γ} ? The structural results point to a critical role
played by the adiacent cysteines ACh convert α_{δ} into the activated conformation resembling α_{γ} ? The structural results point to a critical role played by the adjacent cysteines, α Cys192 and α Cys193, which are at the periphery of the bind bling α_{γ} ? The structural results point to a critical role played by the adjacent cysteines, α Cysl92 and α Cysl93, which are at the periphery of the binding site close to the δ – α -subunit interface (K30 et played by the adjacent cysteines, α Cysl92 and α Cysl93, th
which are at the periphery of the binding site close to the
 δ - α -subunit interface (Kao *et al.* 1984; Czajkowski & cc
Karlin 1995) Arthur Karlin and hi which are at the periphery of the binding site close to the δ - α -subunit interface (Kao *et al.* 1984; Czajkowski & Karlin 1995). Arthur Karlin and his colleagues showed that these cysteines are cross-linked to each δ - α -subunit interface (Kao *et al.* 1984; Czajkowski & Karlin 1995). Arthur Karlin and his colleagues showed that these cysteines are cross-linked to each other, forcing

nance (Williamson *et al.* 2000). Stabilizing the less bent *cis* conformation which, in turn,

The stretch of polypeptide immediately preceding M1 would resist the constraint imposed by the δ-subunit to
 $\lceil (Torpedo \alpha 166$ a non-planar *cis*, rather than the normal *trans* peptide a non-planar *cis*, rather than the normal *trans* peptide
bond. They suggested that the binding of ACh may
initiate a conversion between two stable conformations of a non-planar *cis*, rather than the normal *trans* peptide
bond. They suggested that the binding of ACh may
initiate a conversion between two stable conformations of
the disulphide and peptide bonds (Chandrasekaran $\&$ bond. They suggested that the binding of ACh may
initiate a conversion between two stable conformations of
the disulphide and peptide bonds (Chandrasekaran &
Balasuhramanian 1969) so that Cysl92–193 acts as a initiate a conversion between two stable conformations of
the disulphide and peptide bonds (Chandrasekaran &
Balasubramanian 1969), so that Cys192–193 acts as a
molecular switch controlling receptor activation (Kao & the disulphide and peptide bonds (Chandrasekaran & Balasubramanian 1969), so that Cys192-193 acts as a molecular switch controlling receptor activation (Kao & Karlin 1986). How might this switch trigger the observed molecular switch controlling receptor activation (Kao & Karlin 1986). How might this switch trigger the observed structural change? As figure 11 shows, the alternative *cis* conformations make different bends in the polype Karlin 1986). How might this switch trigger the observed
structural change? As figure 11 shows, the alternative *cis*
conformations make different bends in the polypeptide
chain. Entry of an ACh molecule into the tighter structural change? As figure 11 shows, the alternative *cis*
conformations make different bends in the polypeptide
chain. Entry of an ACh molecule into the tighter α_{δ} -
pocket (figure 10*a*) might therefore trigger t conformations make different bends in the polypeptide chain. Entry of an ACh molecule into the tighter α_{δ} chain. Entry of an ACh molecule into the tighter α_{δ} -
pocket (figure 10*a*) might therefore trigger the change by
stabilizing the less bent *cis* conformation which, in turn,
would resist the constraint imposed by th pocket (figure 10*a*) might therefore trigger the change by stabilizing the less bent *cis* conformation which, in turn, would resist the constraint imposed by the δ -subunit to produce a more extended configuration of produce a more extended configuration of the polypeptide chain. duce a more extended configuration of the polypeptide
in.
A comparison of the α_{δ} and α_{γ} polypeptide configura-
as in the ACb-binding region, before activation, gives

chain.
A comparison of the α_{δ} and α_{γ} polypeptide configura-
tions in the ACh-binding region, before activation, gives
some insight, into the possible physical nature of this A comparison of the α_{δ} and α_{γ} polypeptide configura-
tions in the ACh-binding region, before activation, gives
some insight into the possible physical nature of this
switch. Figure 12 shows the two chains supe tions in the ACh-binding region, before activation, gives
some insight into the possible physical nature of this
switch. Figure 12 shows the two chains superimposed
(after rotating one subunit by 144° relative to the some insight into the possible physical nature of this switch. Figure 12 shows the two chains superimposed (after rotating one subunit by 144° relative to the other). switch. Figure 12 shows the two chains superimposed
(after rotating one subunit by 144° relative to the other).
Assuming now that the α_{γ} configuration is a good
approximation to activated α_{γ} the structura (after rotating one subunit by 144° relative to the other).
Assuming now that the α_{γ} configuration is a good
approximation to activated α_{δ} , the structural change
would entail some reorientation of the α -bel Assuming now that the α_{γ} configuration is a good
approximation to activated α_{δ} , the structural change
would entail some reorientation of the α -helix and some
displacement of the B-hairnin and the rest of th approximation to activated α_{δ} , the structural change
would entail some reorientation of the α -helix and some
displacement of the β -hairpin and the rest of the chain. would entail some reorientation of the α -helix and some displacement of the β -hairpin and the rest of the chain.
In fact, a small overall (clockwise) twist is produced, which might be the origin of the subunit rotat displacement of the β -hairpin and the rest of the chain.
In fact, a small overall (clockwise) twist is produced,
which might be the origin of the subunit rotation detected
earlier (figure 7). Most significant is the ca which might be the origin of the subunit rotation detected earlier (figure 7). Most significant is the ca 3 Å displacewhich might be the origin of the subunit rotation detected
earlier (figure 7). Most significant is the *ca*. 3 Å displace-
ment near the pair of cysteine residues, which is approxi-
mately in the direction (arrow) that wo earlier (figure 7). Most significant is the *ca*. 3 Å displacement near the pair of cysteine residues, which is approximately in the direction (arrow) that would counter the distortion imposed by the δ -subunit ment near the pair of cysteine residue
mately in the direction (arrow) that
distortion imposed by the δ -subunit.
The extracellular (C-terminal) end mately in the direction (arrow) that would counter the distortion imposed by the δ -subunit.
The extracellular (C-terminal) end of the M2 segment

distortion imposed by the δ -subunit.
The extracellular (C-terminal) end of the M2 segment
lies close in projection to the pair of cysteines (figure 12)
and therefore, so does the loop $M2-M3$ implicated in The extracellular (C-terminal) end of the M2 segment
lies close in projection to the pair of cysteines (figure 12)
and, therefore, so does the loop, M2⁻M3, implicated in
the control of gating (Grosman *et al.* 2000). It lies close in projection to the pair of cysteines (figure 12) and, therefore, so does the loop, M2–M3, implicated in the control of gating (Grosman *et al.* 2000). It is plausible then that this displacement initiated near and, therefore, so does the loop, $M2-M3$, implicated in the control of gating (Grosman *et al.* 2000). It is plausible then that this displacement initiated near the pair of cysteines would be transmitted to the M2 segment and move it away from the axis of the pore. Although the concerted transition to onen the channel involves struccysteines would be transmitted to the M2 segment and
move it away from the axis of the pore. Although the
concerted transition to open the channel involves struc-
tural changes in all five subunits (Huwin 1995) not all move it away from the axis of the pore. Although the concerted transition to open the channel involves structural changes in all five subunits (Unwin 1995), not all M₂ segments need directed motion to destabilize the gat concerted transition to open the channel involves structural changes in all five subunits (Unwin 1995), not all M2 segments need directed motion to destabilize the gate and favour the open configuration around the pore. A tural changes in all five subunits (Unwin 1995), not all
M2 segments need directed motion to destabilize the gate
and favour the open configuration around the pore. A
structural link between the binding site and gate, like M2 segments need directed motion to destabilize the gate and favour the open configuration around the pore. A structural link between the binding site and gate, like that and favour the open configuration around the pore. A
structural link between the binding site and gate, like that
depicted in figure 12, might therefore function as a
"communication arm" exerting physiological control by structural link between the binding site and gate, like that
depicted in figure 12, might therefore function as a
'communicating arm', exerting physiological control by
modulating the relative stabilities of the closed and depicted in figure 12, might therefore function as a 'communicating arm', exerting physiological control by modulating the relative stabilities of the closed and open configurations in the membrane 'communicating arm', exerting physiological control by modulating the relative stabilities of the closed and open configurations in the membrane.

13. PORE-OPENING MECHANISM

The earlier electron microscopical experiments high-IS. PORE-OPENING MECHANISM
lighted the role of the M2 segments, not only as the main
structural elements lining the pore, but also as dynamic The earlier electron microscopical experiments high-
lighted the role of the M2 segments, not only as the main
structural elements lining the pore, but also as dynamic
elements effecting its opening or closure. Apparently lighted the role of the M2 segments, not only as the main
structural elements lining the pore, but also as dynamic
elements effecting its opening or closure. Apparently,
these segments move largely independently of the out structural elements lining the pore, but also as dynamic
elements effecting its opening or closure. Apparently,
these segments move largely independently of the outer
linid-facing rim of protein (figure 5) and switch from elements effecting its opening or closure. Apparently,
these segments move largely independently of the outer
lipid-facing rim of protein (figure 5) and switch from one
mode of side-to-side association to the other control these segments move largely independently of the outer
lipid-facing rim of protein (figure 5) and switch from one
mode of side-to-side association to the other, controlled
by the binding of ACh. In the closed-channel form lipid-facing rim of protein (figure 5) and switch from one mode of side-to-side association to the other, controlled
by the binding of ACh. In the closed-channel form of the
receptor, the segments come together near the middle of
the membrane to make the gate of the channel and by the binding of ACh. In the closed-channel form of the receptor, the segments come together near the middle of the membrane to make the gate of the channel and prevent ion permeation. In the open-channel form they receptor, the segments come together near the middle of
the membrane to make the gate of the channel and
prevent ion permeation. In the open-channel form they
come together near the cytoplasmic membrane surface to the membrane to make the gate of the channel and
prevent ion permeation. In the open-channel form they
come together near the cytoplasmic membrane surface to
make the constriction zone, where direct interaction prevent ion permeation. In the open-channel form they
come together near the cytoplasmic membrane surface to
make the constriction zone, where direct interaction
between, the hydrated ions, and encircling protein come together near the cytoplasmic membrane surface to make the constriction zone, where direct interaction between the hydrated ions and encircling protein

BIOLOGICAL
SCIENCES

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BIOLOGICAL
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PHILOSOPHICAL
TRANSACTIONS

determines most critically whether or not the ions can go
through While we do not understand yet the structural determines most critically whether or not the ions can go
through. While we do not understand yet the structural
switch, controlling, the relative stabilities of these two determines most critically whether or not the ions can go
through. While we do not understand yet the structural
switch controlling the relative stabilities of these two
configurations the higher resolution details point t through. While we do not understand yet the structural
switch controlling the relative stabilities of these two
configurations, the higher resolution details point to the
importance of the constraints around the ACh-bindin switch controlling the relative stabilities of these two
configurations, the higher resolution details point to the
importance of the constraints around the ACh-binding
pockets which are relaxed in the open-channel form an configurations, the higher resolution details point to the importance of the constraints around the ACh-binding pockets, which are relaxed in the open-channel form, and importance of the constraints around the ACh-binding
pockets, which are relaxed in the open-channel form, and
the possibility of a direct link between the binding pocket
and M? pockets, with
the possibi
and M2.
The be Expossibility of a direct link between the binding pocket
d M2.
The bend, or kink, near the middle of the M2
rment seems to play a crucial role in facilitating the

and M2.
The bend, or kink, near the middle of the M2
segment, seems to play a crucial role in facilitating the
rapid switch between the closed and open pore. In acting The bend, or kink, near the middle of the M2
segment, seems to play a crucial role in facilitating the
rapid switch between the closed and open pore. In acting
as a point of flexure it allows this critical part of the segment, seems to play a crucial role in facilitating the
rapid switch between the closed and open pore. In acting
as a point of flexure, it allows this critical part of the
receptor to move economically within the rest of rapid switch between the closed and open pore. In acting
as a point of flexure, it allows this critical part of the
receptor to move economically within the rest of the
membrane-spanning structure, so that there is no dire as a point of flexure, it allows this critical part of the receptor to move economically within the rest of the membrane-spanning structure, so that there is no direct receptor to move economically within the rest of the
membrane-spanning structure, so that there is no direct
influence on the motion by the surrounding lipids. The
hend also gives the segment freedom to move towards or membrane-spanning structure, so that there is no direct
influence on the motion by the surrounding lipids. The
bend also gives the segment freedom to move towards or
away from the central axis, and so modifies instantly th influence on the motion by the surrounding lipids. The
bend also gives the segment freedom to move towards or
away from the central axis, and so modifies instantly the
shape size and chemical surroundings of the pore Inter bend also gives the segment freedom to move towards or
away from the central axis, and so modifies instantly the
shape, size and chemical surroundings of the pore. Inter-
estingly molecular dynamics simulations of isolated away from the central axis, and so modifies instantly the shape, size and chemical surroundings of the pore. Interestingly, molecular dynamics simulations of isolated α -belices in water show that those composed of the shape, size and chemical surroundings of the pore. Interestingly, molecular dynamics simulations of isolated α -helices in water show that those composed of the M2 stretch of amino acids have a propensity for localized $\mathbf S$ estingly, molecular dynamics simulations of isolated α -helices in water show that those composed of the M2
stretch of amino acids have a propensity for localized
flexure in their middle portion. The M2 helix unfolds in α -helices in water show that those composed of the M2 stretch of amino acids have a propensity for localized flexure in their middle portion. The M2 helix unfolds in this region in the simulations allowing the bend to stretch of amino acids have a propensity for localized flexure in their middle portion. The M2 helix unfolds in this region, in the simulations, allowing the bend to act as a molecular swivel, or hinge (Law *et al.* 2000) flexure in their middle portion. The M2 helix unfolds in ក

This kind of localized movement within the membrane- spanning part of a structure is presumably This kind of localized movement within the
membrane-spanning part of a structure is presumably
used also by other neurotransmitter-gated ion channels of
the ACh recentor family to alter the dimensions of their membrane-spanning part of a structure is presumably
used also by other neurotransmitter-gated ion channels of
the ACh receptor family to alter the dimensions of their
pores and may indeed be a central feature of the activa used also by other neurotransmitter-gated ion channels of
the ACh receptor family to alter the dimensions of their
pores, and may indeed be a central feature of the activa-
tion mechanisms of many proteins involved in tran the ACh receptor family to alter the dimensions of their
pores, and may indeed be a central feature of the activa-
tion mechanisms of many proteins involved in transporting ions across the lipid bilayer. Bacteriorhodopsin, a tion mechanisms of many proteins involved in trans-
porting ions across the lipid bilayer. Bacteriorhodopsin, a
light-driven proton pump, is an example of a well-studied
membrane protein where flexure of α -belices abou porting ions across the lipid bilayer. Bacteriorhodopsin, a
light-driven proton pump, is an example of a well-studied
membrane protein where flexure of α -helices about a
point near the middle of the membrane has been s light-driven proton pump, is an example of a well-studied
membrane protein where flexure of α -helices about a
point near the middle of the membrane has been shown
to play an important functional role (Subramaniam & membrane protein where flexure of α -helices about a channel involves large changes in pore dimensions, the point near the middle of the membrane has been shown accompanying conformational change should be detect-
to pla point near the middle of the membrane has been shown

14. RELATED EVENTS AT THE RELATED EVENTS AT THE
ELECTRICAL SYNAPSE

ELECTRICAL SYNAPSE
The electrical synapse is a region of contact between a pair of nerve cells that mediates fast electrical trans-The electrical synapse is a region of contact between a
pair of nerve cells that mediates fast electrical trans-
mission by providing pathways for ion diffusion through
connecting gan junction channels. Although less commo pair of nerve cells that mediates fast electrical transmission by providing pathways for ion diffusion through connecting gap junction channels. Although less common in the nervous system than the chemical syname it is mission by providing pathways for ion diffusion through
connecting gap junction channels. Although less common
in the nervous system than the chemical synapse, it is
being found in many different parts with increasing connecting gap junction channels. Although less common
in the nervous system than the chemical synapse, it is
 \rightarrow being found in many different parts with increasing
 \rightarrow frequency (Bennett 2000). in the nervous system than
being found in many dif
frequency (Bennett 2000).
The gap junction channe ing found in many different parts with increasing
quency (Bennett 2000).
The gap junction channel is built from a ring of six
nilar, or identical protein subunits, called connexins

The gap junction channel is built from a ring of six
similar, or identical protein subunits, called connexins. The connexins constitute a family of homologous polysimilar, or identical protein subunits, called connexins.
The connexins constitute a family of homologous poly-
peptides that fold to form four- α -helical bundle structures
within the linid bilayer (Unger *et al.* 1999) The connexins constitute a family of homologous poly-
peptides that fold to form four- α -helical bundle structures
within the lipid bilayer (Unger *et al.* 1999). They are
unusual because of their dual role; not only do peptides that fold to form four- α -helical bundle structures
within the lipid bilayer (Unger *et al.* 1999). They are
unusual because of their dual role: not only do they
associate within the plasma membrane of one cell within the lipid bilayer (Unger *et al.* 1999). They are unusual because of their dual role: not only do they associate within the plasma membrane of one cell to make a channel but they also join to connexing in the unusual because of their dual role: not only do they
associate within the plasma membrane of one cell to
make a channel, but they also join to connexins in the
plasma membrane of another cell to make a continuous associate within the plasma membrane of one cell to make a channel, but they also join to connexins in the plasma membrane of another cell to make a continuous conducting pathway between the two interiors. A widely make a channel, but they also join to connexins in the
plasma membrane of another cell to make a continuous
conducting pathway between the two interiors. A widely
expressed 36kDa neuron-specific connexin has recently plasma membrane of another cell to make a continuous
conducting pathway between the two interiors. A widely
expressed, 36 kDa neuron-specific connexin has recently
been cloned (Condorelli et al. 1998: Söhl et al. 1998) conducting pathway between the two interiors. A wisexpressed, 36 kDa neuron-specific connexin has recessioned (Condorelli *et al.* 1998; Söhl *et al.* 1998). Gan iunction channels have not been explored in expressed, 36kDa neuron-specific connexin has recently been cloned (Condorelli *et al.* 1998; Söhl *et al.* 1998). Gap junction channels have not been explored in the

been cloned (Condorelli *et al.* 1998; Söhl *et al.* 1998).
Gap junction channels have not been explored in the same detail as the ACh receptor; however, the studies so far carried out suggest that the two channels may ha Gap junction channels have not been explored in the
same detail as the ACh receptor; however, the studies so
far carried out suggest that the two channels may have
structural parallels and work by similar physical same detail as the ACh receptor; however, the studies so
far carried out suggest that the two channels may have
structural parallels and work by similar physical *Phil. Trans. R. Soc. Lond.* B (2000)
Phil. Trans. R. Soc. Lond. B (2000)

Figure 13. Model for the concerted transition between closed
and open forms of the gap junction channel, based on low
resolution electron crystallographic studies. Tilting of the and open forms of the gap junction channel, based on low
resolution electron crystallographic studies. Tilting of the protein subunits around the axis of the channel gives rise to resolution electron crystallographic studies. Tilting of the
protein subunits around the axis of the channel gives rise to
small tangential displacements in the plane of the membrane,
which combine to make large changes in protein subunits around the axis of the channel gives rise to
small tangential displacements in the plane of the membrane,
which combine to make large changes in pore dimensions at
the cytoplasmic membrane surface (adanted small tangential displacements in the plane of the membrane,
which combine to make large changes in pore dimensions at
the cytoplasmic membrane surface (adapted from Unwin &
Zampighi 1980) which combine to 1
the cytoplasmic me
Zampighi 1980).

principles. The putative pore-lining segment, M3, has a principles. The putative pore-lining segment, M3, has a
similar pattern of bulky hydrophobic and small polar
residues as M2 of the ACh recentor, and there is a principles. The putative pore-lining segment, M3, has a similar pattern of bulky hydrophobic and small polar residues as M2 of the ACh receptor, and there is a conserved phenylalanine in place of $\alpha I \text{ e}^{1251}$ (Unwing) similar pattern of bulky hydrophobic and small polar residues as M2 of the ACh receptor, and there is a conserved phenylalanine in place of α Leu251 (Unwin 1989) But the gap iunction pore is wider and less selection residues as M2 of the ACh receptor, and there is a
conserved phenylalanine in place of α Leu251 (Unwin
1989). But the gap junction pore is wider and less selec-
tive allowing the passage of small molecules (ϵ)conserved phenylalanine in place of α Leu251 (Unwin 1989). But the gap junction pore is wider and less selective, allowing the passage of small molecules ($<$ 1-1989). But the gap junction pore is wider and less selective, allowing the passage of small molecules $(<1-2kDa$), as well as ions, between connected cells. Also gap junction channels do not desensitize making it possible t tive, allowing the passage of small molecules $(<1-2kDa$), as well as ions, between connected cells. Also gap junction channels do not desensitize, making it possible to stabilize either the closed- or open-channel form sim 2 kDa), as well as ions, between connected cells. Also gap
junction channels do not desensitize, making it possible to
stabilize either the closed- or open-channel form simply
by using different concentrations of ligand. H junction channels do not desensitize, making it possible to stabilize either the closed- or open-channel form simply by using different concentrations of ligand. High concenstabilize either the closed- or open-channel form simply
by using different concentrations of ligand. High concentrations of Ca^{2+} and H^+ ions lead to rapid closure (Rose
& Loewenstein 1977: Spray *et al* 1981) by using different concentrations of ligations of Ca^{2+} and H^+ ions lead to ratio & Loewenstein 1977; Spray *et al.* 1981).
Since the gating mechanism of the tions of Ca^{2+} and H^{+} ions lead to rapid closure (Rose
Loewenstein 1977; Spray *et al.* 1981).
Since the gating mechanism of the gap junction
annel involves large changes in pore dimensions the

& Loewenstein 1977; Spray *et al.* 1981).
Since the gating mechanism of the gap junction
channel involves large changes in pore dimensions, the
accompanying conformational change should be detect-Since the gating mechanism of the gap junction
channel involves large changes in pore dimensions, the
accompanying conformational change should be detect-
able even at low resolution. Electron crystallographic channel involves large changes in pore dimensions, the
accompanying conformational change should be detect-
able even at low resolution. Electron crystallographic
studies (Hnwin & Zampighi 1980) and low-angle X-ray accompanying conformational change should be detect-
able even at low resolution. Electron crystallographic
studies (Unwin & Zampighi 1980) and low-angle X-ray
diffraction experiments (Unwin & Ennis 1983) able even at low resolution. Electron crystallographic
studies (Unwin & Zampighi 1980) and low-angle X-ray
diffraction experiments (Unwin & Ennis 1983),
conducted on isolated gan junctions, have shown that studies (Unwin & Zampighi 1980) and low-angle X-ray
diffraction experiments (Unwin & Ennis 1983),
conducted on isolated gap junctions, have shown that
there are indeed two alternative arrangements of the diffraction experiments (Unwin & Ennis 1983), conducted on isolated gap junctions, have shown that there are indeed two alternative arrangements of the conducted on isolated gap junctions, have shown that
there are indeed two alternative arrangements of the
protein subunits around the pore (figure 13). At high
 $[Ca^{2+1}]$ where the channel should be closed the subunits $[Ca^{2+}]$, where the channel should be closed, the subunits are indeed two alternative arrangements of the
in subunits around the pore (figure 13). At high
], where the channel should be closed, the subunits
rientated approximately parallel to the pore axis: protein subunits around the pore (figure 13). At high $[Ca^{2+}]$, where the channel should be closed, the subunits are orientated approximately parallel to the pore axis; while at low $[Ca^{2+}]$ where the channel should be op [Ca²⁺], where the channel should be closed, the subunits
are orientated approximately parallel to the pore axis;
while at low [Ca²⁺], where the channel should be open,
they are inclined tangentially to this axis. The are orientated approximately parallel to the pore axis;
while at low $[\text{Ca}^{2+}]$, where the channel should be open,
they are inclined tangentially to this axis. The lowered
 $[\text{Ca}^{2+}]$ widens the pore at the cytoplasmic m [Ca²⁺] widen
surface in pro
Ennis 1984).
The gap juy at low $[Ca^{2+}]$, where the channel should be open,
are inclined tangentially to this axis. The lowered
] widens the pore at the cytoplasmic membrane
is in proportion to this change in tilt (Unwin & they are inclined tangentially to this axis. The lowered $[Ca^{2+}]$ widens the pore at the cytoplasmic membrane surface in proportion to this change in tilt (Unwin & Ennis 1984). rface in proportion to this change in tilt (Unwin &
nnis 1984).
The gap junction and the ACh receptor channels there-
se both use coordinated tilting of α -belical segments

Ennis 1984).
The gap junction and the ACh receptor channels there-
fore both use coordinated tilting of α -helical segments
around the pore to bring about changes in its properties The gap junction and the ACh receptor channels therefore both use coordinated tilting of α -helical segments around the pore to bring about changes in its properties.
In both cases the tilt axes lie approximately parall fore both use coordinated tilting of α -helical segments
around the pore to bring about changes in its properties.
In both cases the tilt axes lie approximately parallel to
the membrane plane creating displacements pred around the pore to bring about changes in its properties.
In both cases the tilt axes lie approximately parallel to
the membrane plane, creating displacements predomi-
nantly within the plane that keep the same polar and In both cases the tilt axes lie approximately parallel to the membrane plane, creating displacements predominantly within the plane that keep the same polar and the membrane plane, creating displacements predominantly within the plane that keep the same polar and hydrophobic surfaces exposed to water, protein and lipid.
This kind of rearrangement common to both channels is nantly within the plane that keep the same polar and
hydrophobic surfaces exposed to water, protein and lipid.
This kind of rearrangement, common to both channels, is
energetically favourable and efficient in terms of mini hydrophobic surfaces exposed to water, protein and lipid.
This kind of rearrangement, common to both channels, is
energetically favourable and efficient in terms of minim-
izing perturbation of the structure to achieve max This kind of rearrangement, common to both channels, is
energetically favourable and efficient in terms of minim-
izing perturbation of the structure to achieve maximum energetically favourable and efficient in terms of minimizing perturbation of the structure to achieve maximum effect. Small in-plane displacements of individual α -helices or subunits around a pore combine to alter sig effect. Small in-plane displacements of individual α helices or subunits around a pore combine to alter signifithey expose different sets of amino-acid side-chains and/
or backbone groups to the lumen and introduce new
physically and chemically distinct surfaces. or backbone groups to the lumen and introduce new

PHILOSOPHICAL
TRANSACTIONS

A recent analysis by electron paramagnetic resonance spectroscopy of the KscA channel (Perozo *et al.* 1999), spectroscopy of the KscA channel (Perozo *et al.* 1999), phase water-lipid environment. Both channels use co-
suggested that the membrane-spanning α -helices of K⁺ ordinated lateral motions in the membrane, developed spectroscopy of the KscA channel (Perozo *et al.* 1999), suggested that the membrane-spanning α -helices of K⁺ channels may move according to these principles, upon activation to change the properties of the pore. The suggested that the membrane-spanning α -helices of K^+
channels may move according to these principles, upon
activation, to change the properties of the pore. The same
might therefore he said about the synantic channe channels may move according to these principles, upon
activation, to change the properties of the pore. The same
might therefore be said about the synaptic channels gated
by glutamate which are related in sequence to K^+ activation, to change the properties of the pore. The
might therefore be said about the synaptic channels β
by glutamate, which are related in sequence to K $^+$ channels
(Chen *et al* 1999) by glutamate, which are related in sequence to K^+ channels might therefore be said about the synaptic channels gated
by glutamate, which are related in sequence to K^+ channels
(Chen *et al.* 1999).

15. CONCLUSIONS

Release of chemical transmitter at the nerve terminal **leads** to a fast, predictable electrical response at the newslet of the target cell which is Release of chemical transmitter at the nerve terminal
leads to a fast, predictable electrical response at the
postsynaptic membrane of the target cell, which is
mediated by the rapid and robust action of ion-selective postsynaptic membrane of the target cell, which is H mediated by the rapid and robust action of ion-selective postsynaptic membrane of the target cell, which is
mediated by the rapid and robust action of ion-selective
neurotransmitter-gated channels. Many of the principles
underlying the behaviour of these channels are probably mediated by the rapid and robust action of ion-selective
neurotransmitter-gated channels. Many of the principles
underlying the behaviour of these channels are probably
quite general and shared with the ACh recentor the on neurotransmitter-gated channels. Many of the principles
underlying the behaviour of these channels are probably
quite general and shared with the ACh receptor, the only
neurotransmitter-gated channel for which a 3D structu underlying the behaviour of these channels are probably
quite general and shared with the ACh receptor, the only
neurotransmitter-gated channel for which a 3D structure

quite general and shared with the ACh receptor, the only
neurotransmitter-gated channel for which a 3D structure
is available. The structures I have described of this
protein suggest that interaction of neurotransmitter wi neurotransmitter-gated channel for which a 3D structure
is available. The structures I have described of this
protein suggest that interaction of neurotransmitter with
its receptor sets in train a number of events involvin is available. The structures I have described of this
protein suggest that interaction of neurotransmitter with
its receptor sets in train a number of events, involving all
subunits, which are distributed over the length o protein suggest that interaction of neurotransmitter with
its receptor sets in train a number of events, involving all $\frac{1}{2}$ channel. subunits, which are distributed over the length of the channel.
The ACh receptor-mediated events might be summar-

channel.
The ACh receptor-mediated events might be summarized as follows. ACh molecules are drawn electrostatically
into the mouth of the extracellular vestibule and selec-The ACh receptor-mediated events might be summarized as follows. ACh molecules are drawn electrostatically
into the mouth of the extracellular vestibule and selec-
tively guided through tunnels in the wall to pockets ized as follows. ACh molecules are drawn electrostatically
into the mouth of the extracellular vestibule and selec-
tively guided through tunnels in the wall to pockets
located within both α -subunits. The free energy o into the mouth of the extracellular vestibule and selectively guided through tunnels in the wall to pockets located within both α -subunits. The free energy of binding then triggers a concerted allosteric transition by tively guided through tunnels in the wall to pockets
located within both α -subunits. The free energy of
binding then triggers a concerted allosteric transition by
a mechanism in which the two adjacent α -subunit located within both α -subunits. The free energy of binding then triggers a concerted allosteric transition by a mechanism in which the two adjacent α -subunit binding then triggers a concerted allosteric transition by
a mechanism in which the two adjacent α -subunit
cysteines may play a key role. All subunits are affected,
enabling the α -subunits to overcome the constraint a mechanism in which the two adjacent α -subunit
cysteines may play a key role. All subunits are affected,
enabling the α -subunits to overcome the constraints
imposed on them by neighbouring subunits and become cysteines may play a key role. All subunits are affected,
enabling the α -subunits to overcome the constraints
imposed on them by neighbouring subunits and become
more nearly equivalent. The conformational change enabling the α -subunits to overcome the constraints
imposed on them by neighbouring subunits and become
more nearly equivalent. The conformational change produces a twisting of the α -subunit between the binding more nearly equivalent. The conformational change
produces a twisting of the α -subunit between the binding
pocket and the membrane, which is communicated to the
membrane-spanning segment M_2 drawing it away from produces a twisting of the α -subunit between the binding
pocket and the membrane, which is communicated to the
membrane-spanning segment, M2, drawing it away from
the lumen of the pore. This weakens the stability of th pocket and the membrane, which is communicated to the
membrane-spanning segment, M2, drawing it away from
the lumen of the pore. This weakens the stability of the
hydrophobic girdle forming the gate near the middle of membrane-spanning segment, M2, drawing it away from
the lumen of the pore. This weakens the stability of the
hydrophobic girdle, forming the gate near the middle of
the membrane in favour of the alternative 'onen' conthe lumen of the pore. This weakens the stability of the
hydrophobic girdle, forming the gate near the middle of
the membrane, in favour of the alternative 'open' con-
figuration of M2 segments where the pore is wider near hydrophobic girdle, forming the gate near the middle of
the membrane, in favour of the alternative 'open' con-
figuration of M2 segments where the pore is wider near
the middle of the membrane and most constricted at the the membrane, in favour of the alternative 'open' con-
figuration of M2 segments where the pore is wider near
the middle of the membrane and most constricted at the
extends micropheanic membrane surface. The selected ions figuration of M2 segments where the pore is wider near
the middle of the membrane and most constricted at the
cytoplasmic membrane surface. The selected ions now
pass readily across the membrane because the pore has a the middle of the membrane and most constricted at the cytoplasmic membrane surface. The selected ions now pass readily across the membrane because the pore has a short constriction lined by polar residues instead of the cytoplasmic membrane surface. The selected ions now
pass readily across the membrane because the pore has a
short constriction lined by polar residues, instead of the
hydrophobic barrier and because ions of the right charg pass readily across the membrane because the pore has a
short constriction lined by polar residues, instead of the
hydrophobic barrier, and because ions of the right charge
have been concentrated relative to those of the w short constriction lined by polar residues, instead of the hydrophobic barrier, and because ions of the right charge
have been concentrated relative to those of the wrong
charge by preselection through the vestibules Initi \Box hydrophobic barrier, and because ions of the right charge \Box have been concentrated relative to those of the wrong
 \Box charge by preselection through the vestibules. Initially, have been concentrated relative to those of the wrong
charge by preselection through the vestibules. Initially,
the driving force is mainly for $Na⁺$ ions from the outside
to flow into the cell but as the membrane dep charge by preselection through the vestibules. Initially,
the driving force is mainly for Na^+ ions from the outside
to flow into the cell but, as the membrane depolarizes, an
increasing proportion of K^+ ions flow in the driving force is mainly for Na^+ ions from the outside
to flow into the cell but, as the membrane depolarizes, an
increasing proportion of K^+ ions flow in the opposite
direction. Soon, after a millisecond or so, t to flow into the cell but, as the membrane depolarizes, an increasing proportion of K^+ ions flow in the opposite direction. Soon, after a millisecond or so, the weakly bound ACh melecules are released from their bindi increasing proportion of K^+ ions flow in the opposite
direction. Soon, after a millisecond or so, the weakly
bound ACh molecules are released from their binding
pockets into the now ACh-denleted surroundings the direction. Soon, after a millisecond or so, the weakly
bound ACh molecules are released from their binding
pockets into the now ACh-depleted surroundings, the
transient open-channel structure is no longer favoured bound ACh molecules are released from their binding
pockets into the now ACh-depleted surroundings, the
transient open-channel structure is no longer favoured
and the receptor returns to its original pop-conducting pockets into the now ACh-depleted surroundings, the transient open-channel structure is no longer favoured and the receptor returns to its original non-conducting state. d the receptor returns to its original non-conducting
I have also described briefly some properties of the gap
action channel the relatively non-selective channel

state.

I have also described briefly some properties of the gap

junction channel, the relatively non-selective channel

mediating fast electrical synaptic transmission. The struc-I have also described briefly some properties of the gap
junction channel, the relatively non-selective channel
mediating fast electrical synaptic transmission. The struc-
tural rearrangements around the pore of this prote junction channel, the relatively non-selective channel
mediating fast electrical synaptic transmission. The struc-
tural rearrangements around the pore of this protein were
seen to be analogous to those of the receptor ent mediating fast electrical synaptic transmission. The structural rearrangements around the pore of this protein were
seen to be analogous to those of the receptor, entailing *Phil. Trans. R. Soc. Lond.* B (2000)

changes that can readily be accommodated in the two changes that can readily be accommodated in the two
phase water-lipid environment. Both channels use co-
ordinated lateral motions in the membrane, developed by changes that can readily be accommodated in the two
phase water–lipid environment. Both channels use co-
ordinated lateral motions in the membrane, developed by
tilting of α -belical segments to effect opening and phase water–lipid environment. Both channels use co-
ordinated lateral motions in the membrane, developed by
tilting of α -helical segments, to effect opening and
closure of the nore. It would not be surprising if all f ordinated lateral motions in the membrane, developed by
tilting of α -helical segments, to effect opening and
closure of the pore. It would not be surprising if all fast
synantic transmission is mediated by this same ge tilting of α -helical segments, to effect opening and closure of the pore. It would not be surprising if all fast synaptic transmission is mediated by this same general mechanism mechanism. naptic transmission is mediated by this same general
echanism.
Despite the progress made over the past 40 years in
sualizing events, and in determining the molecular

mechanism.
Despite the progress made over the past 40 years in
visualizing events and in determining the molecular
properties underlying the fast postsynaptic response, we Despite the progress made over the past 40 years in
visualizing events and in determining the molecular
properties underlying the fast postsynaptic response, we
still only have a fairly elementary nicture through the visualizing events and in determining the molecular
properties underlying the fast postsynaptic response, we
still only have a fairly elementary picture through the
example of the ACh receptor. It would be valuable to properties underlying the fast postsynaptic response, we
still only have a fairly elementary picture through the
example of the ACh receptor. It would be valuable to
know the precise organization of amino-acid side-chains still only have a fairly elementary picture through the example of the ACh receptor. It would be valuable to know the precise organization of amino-acid side-chains in the vicinity of the ACh-binding pockets, so that the example of the ACh-receptor. It would be valuable to
know the precise organization of amino-acid side-chains
in the vicinity of the ACh-binding pockets, so that the
binding reaction could be better understood. Knowledge know the precise organization of amino-acid side-chains
in the vicinity of the ACh-binding pockets, so that the
binding reaction could be better understood. Knowledge
of the folding of the polypentide chains extending from in the vicinity of the ACh-binding pockets, so that the binding reaction could be better understood. Knowledge of the folding of the polypeptide chains extending from the binding region to the membrane would allow a more binding reaction could be better understood. Knowledge
of the folding of the polypeptide chains extending from
the binding region to the membrane would allow a more of the folding of the polypeptide chains extending from
the binding region to the membrane would allow a more
complete account of the coordinated ligand–receptor
action controlling the stability of the gate Insight with the binding region to the membrane would allow a more
complete account of the coordinated ligand–receptor
action controlling the stability of the gate. Insight with
wide implications would be obtained by defining the action controlling the stability of the gate. Insight with wide implications would be obtained by defining the action controlling the stability of the gate. Insight with
wide implications would be obtained by defining the
protein scaffold that partitions the moving pore-lining
helices away from the linids: does this membranewide implications would be obtained by defining the
protein scaffold that partitions the moving pore-lining
helices away from the lipids; does this membrane-
spanning motif entail subunit-subunit interactions protein scaffold that partitions the moving pore-lining
helices away from the lipids; does this membrane-
spanning motif entail subunit-subunit interactions
through the polynentide backbone groups which would helices away from the lipids; does this membrane-
spanning motif entail subunit-subunit interactions
through the polypeptide backbone groups, which would spanning motif entail subunit-subunit interactions
through the polypeptide backbone groups, which would
maintain a constant 3D framework so that alternative
subunits can be precisely 'slotted in'? An exact localization through the polypeptide backbone groups, which would
maintain a constant 3D framework so that alternative
subunits can be precisely 'slotted in'? An exact localization
of charged polar and hydrophobic groups would enable a subunits can be precisely 'slotted in'? An exact localization
of charged, polar and hydrophobic groups would enable a subunits can be precisely 'slotted in'? An exact localization
of charged, polar and hydrophobic groups would enable a
quantitative description of the electrostatic influences on
ion-selectivity and the rate of transport th of charged, polar and hydrophobic groups would enable a
quantitative description of the electrostatic influences on
ion-selectivity and the rate of transport through the pore.
Fortunately an atomic model of the ACh recento quantitative description of the electrostatic influences on
ion-selectivity and the rate of transport through the pore.
Fortunately an atomic model of the ACh receptor seems
not too far away. It should help in extending si ion-selectivity and the rate of transport through the pore.
Fortunately an atomic model of the ACh receptor seems
not too far away. It should help in extending significantly our understanding not only of this ion channel, but also of others at the synap se, and elsewhere.

All the more recent work has been conducted in collaboration
with Professor Yoshi Fujiyoshi, making use of his bighly stable All the more recent work has been conducted in collaboration
with Professor Yoshi Fujiyoshi, making use of his highly stable,
liquid helium-cooled microscone stage. To him I owe special All the more recent work has been conducted in collaboration
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thanks I am also fortunate to have w with Professor Yoshi Fujiyoshi, making use of his highly stable,
liquid helium-cooled microscope stage. To him I owe special
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other outstanding colleagues at the liquid helium-cooled microscope stage. To him I owe special
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other outstanding colleagues at the MRC Laboratory of
Molecular Biology Cambridge IIK the Scripps Res other outstanding colleagues at the MRC Laboratory of Molecular Biology, Cambridge, UK, the Scripps Research other outstanding colleagues at the MRC Laboratory of Molecular Biology, Cambridge, UK, the Scripps Research Institute, La Jolla, CA, USA and Kyoto University, Japan whose advice collaboration and support have strongly Molecular Biology, Cambridge, UK, the Scripps Research
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