Journal of Neuroscience Methods, 30 (1989) 203–210
Elsevier

NSM 01013

Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex

Mark G. Blanton, Joseph J. Lo Turco and Arnold R. Kriegstein

Department of Neurology M016, Stanford University Medical Center, Stanford, CA 94305-5300 (U S A)

(Received 7 March 1989)
(Revised 26 June 1989)
(Accepted 30 June 1989)

Key words: Voltage clamp; Whole cell recording, Cortex; Brain slices; Synaptic currents; Rat; Turtle

We describe methods for obtaining stable, whole-cell recordings from neurons in brain hemispheres from turtles and in brain slices from rats and turtles Synaptic currents and membrane properties of central neurons can be studied in voltage and current clamp in cells maintained within their endogenous synaptic circuits. The methods described here are compatible with unmodified dissecting microscopes and recording chambers, and with brain slices of standard thickness (400–500 μm).

Introduction

Microelectrode recording techniques have been used extensively to measure synaptic and membrane properties of neurons in brain slices (Dingledine, 1984). A limitation of microelectrode methods is the tradeoff between small electrode tip size needed for impaling neurons and the low resistance needed for passing current through the microelectrode. This compromise is largely avoided by the electrodes used in the whole-cell patch-clamp technique (see Sakmann and Neher, 1983). In whole cell recording, a high-resistance (gigaohm) seal is formed between a relatively large electrode tip and a cell membrane, and then the underlying membrane patch is ruptured to produce low resistance electrical access to the cell interior. Whole cell recording from neurons in intact neuronal tissue has been considered impractical, because the extracellular matrix and glial investment of neuronal perikarya were assumed to prevent formation of gigaohm seals between electrodes and cells.

Recent work indicates that, if care is taken to prevent clogging of the patch pipette tip, whole cell recordings can be made in thin slices of mammalian tissue (Konnerth et al., 1988), in thin amphibian retinal slices (Barnes and Werblin, 1986), and in an enzymatically treated eyecup preparation (Coleman and Miller, 1989) Konnerth et al. (1988) used a suction pipette to 'clean' the surface of a visually identified neuron in a tissue slice before forming a seal. Coleman and Miller (1989) maintained an unclogged tip by applying positive pressure through the recording pipette and sealing onto cells below the retinal surface We describe here a simple method for obtaining whole-cell recordings in mammalian and reptilian cerebral cortex that does not require special optics, physical disruption, or enzymatic treatment of tissue. We demonstrate the broad applicability of this procedure with typical recordings in both current- and voltage-clamp in several neuronal preparations.

Correspondence Dr M G Blanton, Dept of Neurology, M016, Stanford University Medical Center, Stanford, CA 94305-5300, U S A

Methods and results

Solutions

Mammalian artificial cerebrospinal fluid (aCSF; Connors et al., 1982) contained (in mM): NaCl, 124; KCl, 5; $MgSO_4$, 2; $CaCl_2$, 2; NaH_2PO_4, 1.25; $NaHCO_3$, 26; dextrose, 10. Turtle aCSF (Mori et al., 1981) contained (in mM): NaCl, 96.5; KCl, 2.6; $MgCl_2$, 2; $CaCl_2$, 2 or 4; $NaHCO_3$ 31.5; dextrose, 10. In some experiments, bicuculline methiodide (Sigma) was added to the bathing aCSF.

Tissue preparation

Rats (Sprague–Dawley) aged P0 to P45 and turtles (*Pseudemys scripta elegans*) at embryonic, hatchling and adult ages were used in these experiments. Animals were anesthetized with hypothermia (rat aged P0-9, embryonic and hatchling turtles) or with an intraperitoneal injection of pentobarbital (50 mg/kg) in older animals (rats P10–45, adult turtles). Cerebral hemispheres were removed, blocked and mounted on the vibratome stage (Lancer) with cyanoacrylate glue (Krazy-glue). Slices (400–500 μm thick) were cut and collected in cooled aCSF (5 °C). To facilitate slicing of the thin cerebral mantle of the turtle, cerebral hemispheres were immersed in warm 3% agar (Difco) in turtle aCSF and then the agar blocks containing the hemispheres were hardened on ice and sliced with a vibratome (200–1500 μm).

The thinness of the turtle cerebral cortex and its resistance to anoxia allow the entire cortical mantle to be removed as a sheet for some experiments, obviating the need for slicing. Rostral, caudal and midline septal cuts of the hemisphere allow the thin (400 μm in hatchling, 800 μm in adult) cerebral cortex to be flattened for recording. Subcortical structures and their connections to the cortex can thus be maintained, or alternatively an incision lateral to dorsal cortex can be used to completely isolate the cortical slab

Tissue stabilization for recording

A fibrin clot (Harrison, 1910; Takahashi, 1978) was used to attach acutely prepared slices and slabs to 35-mm petri dishes for recording (Fig. 1). Tissue was picked up with a spatula, excess aCSF

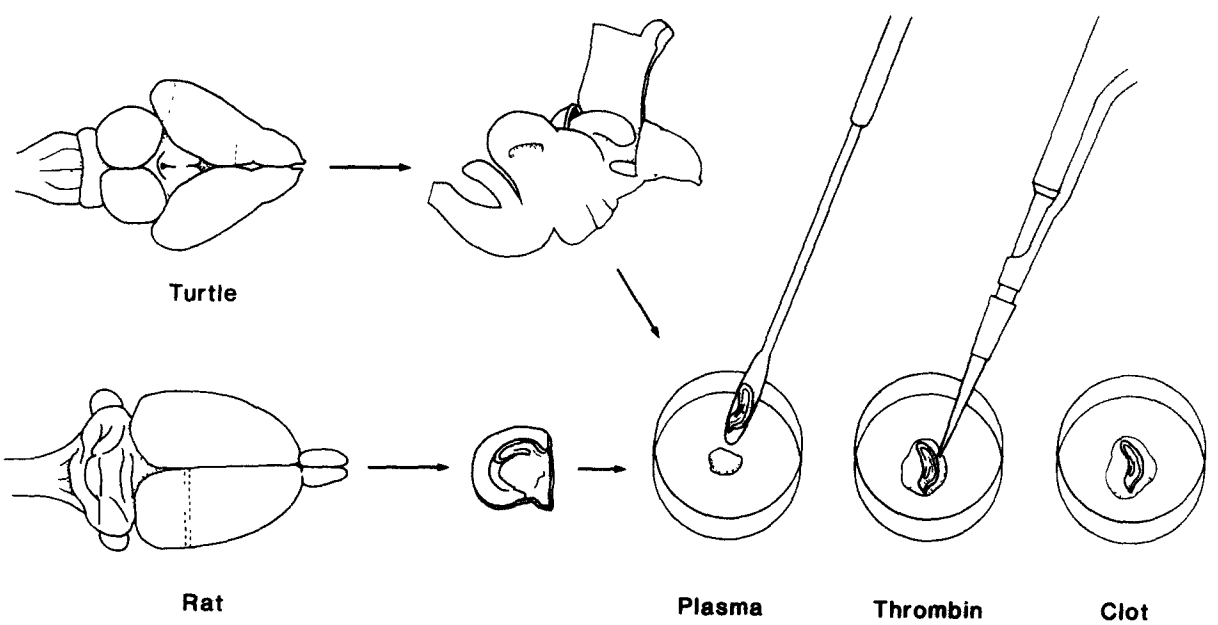

Fig. 1. Schematic illustration of the attachment of tissue preparations to petri dishes for recording. Turtle brain hemispheres or rat or turtle brain slices were placed in a small volume of chicken plasma, and then an equal volume of thrombin was added to form a fibrin clot to hold tissue in place (see text for details)

was blotted away and the tissue gently pushed off the spatula into a small volume (10–15 μl) of chicken plasma (Sigma). An equal volume of bovine thrombin (285 units/ml, Sigma) was added, taking care to mix the thrombin and plasma without coating the tissue surface with the clot. The clot was allowed to form for approx. 20 min in an oxygenated (95% O_2/5% CO_2), humidified environment. Adding fibrinogen (5 μl of a 2 mg/ml solution, Sigma) or tissue thromboplastin after adding thrombin shortened the time needed for clot formation to approx. 10 min. Following clot formation, the petri dish was filled with aCSF and maintained in an oxygenated, humidified environment until used.

Electrodes

Patch electrodes were pulled (one stage pull) from borosilicate glass (WPI, New Haven, CT) on a Kopf vertical puller and had tip resistances of 3-7MΩ. In some experiments, Sigmacoat (Sigma) was applied to the electrode shank to reduce electrode capacitance. Electrodes were filled with one of 3 solutions, containing (in mM): solution 1: potassium methanesulfonate, 110; KCl, 10; HEPES buffer, 10; potassium EGTA, 5; $MgCl_2$, 1; solution 2: CsF, 130; TEA-Cl, 10; NaCl, 5; $MgCl_2$, 1, $CaCl_2$, 1; EGTA, 11; HEPES buffer, 10; or solution 3: CsCl, 120; HEPES buffer, 10; EGTA, 11; $CaCl_2$, 1; $MgCl_2$, 1; TEA, 2, QX-314, 16.7.

In some experiments, a saturated solution of the fluorescent dye lucifer yellow (LY) dipotassium salt (Molecular Probes) was prepared in solutions 2 or 3 and used to fill the tips of electrodes, which were then backfilled with the same solution without LY. Tissue was fixed with phosphate-buffered 4% paraformaldehyde, viewed under fluorescence to verify the LY fill, and then sectioned Sections were incubated with an antiserum against LY (kindly provided by Dr. B. Wong) and further processed using standard histochemical techniques, as described by Taghert et al. (1982).

Bipolar stimulating electrodes were fashioned by gluing together 2 insulated tungsten electrodes (Frederick Haer), with tips approx. 500 μm apart. Stimulating current (100 μsec duration) was applied through the electrodes from a stimulus isolation unit (WPI).

Electronics and data acquisition

Recordings were made using a List EPC 7 patch clamp amplifier. The head stage carrying the electrode was mounted on either a Narashige hydraulic micromanipulator or a Leitz mechanical micromanipulator. Current and voltage data were digitized using a Neuro-corder (model DR-484, Neurodata Instruments) and stored on VCR tape for subsequent analysis. The pCLAMP data acquisition program run on a Hewlett Packard Vectra ES/12 was used to acquire and analyze data on- and off-line.

Obtaining whole cell recordings

The techniques detailed below and illustrated in Fig. 2 allowed whole cell recordings from neurons in turtle and rat cerebral cortex. A petri dish containing neural tissue was placed on the stage of an upright microscope (aus Jena) or in a chamber viewed with a dissecting microscope, and the tissue was superfused with oxygenated aCSF at room temperature (22–25°C). Positive pressure was applied to the back of a recording pipette using a 10-ml syringe connected by polyethylene tubing to the electrode holder, and while maintaining positive pressure, the electrode pipette tip was then passed into the tissue and could be driven to any desired depth. Small voltage steps (1 mV) were applied, and a small decrease in the current deflection (from 20 to 50% of initial amplitude) signalled that the electrode tip was approaching a cell. Slight negative pressure was then applied with the syringe (1–2 ml), frequently resulting in the formation of stable GΩ seals. In this configuration, it was possible to record current from spontaneous action potentials or single channel activity in cell-attached patches. The membrane patch was ruptured to obtain whole-cell recordings by applying additional slight negative pressure and positive voltage steps from a holding potential of −70 mV.

Visual inspection using Hoffman interference contrast optics helped in electrode placement, allowing individual cells and cell layers to be clearly seen, but it was not necessary to select specific individual neurons for recording. We found use of

a low power dissecting microscope sufficient for placing the electrode.

The techniques described above provided a high success rate in recording from central neurons in turtle ($n = 320$) and rat ($n = 270$) brain slices. GΩ seals (usually 2–10 GΩ) were formed and whole cell recordings obtained in more than 90% of attempts in turtle preparations and in approx. 50% of attempts in rat slices.

Recordings in turtle cortex

Recordings could be maintained in both embryonic and mature slices for up to several hours. Cortical neurons in juvenile turtles typically had stable resting potentials (-61.1 ± 11.2 mV, $n = 19$), similar to the values obtained using conventional sharp electrodes in mature animals (Connors and Kriegstein, 1986). In response to current injection, all neurons fired repetitive action potentials (74 ± 13.4 mV in amplitude, $n = 22$), with duration at half-amplitude ranging from 1.5 to 4 msec. The distinct action potential waveforms and firing patterns of pyramidal and nonpyramidal cells (Connors and Kriegstein, 1986), were re-

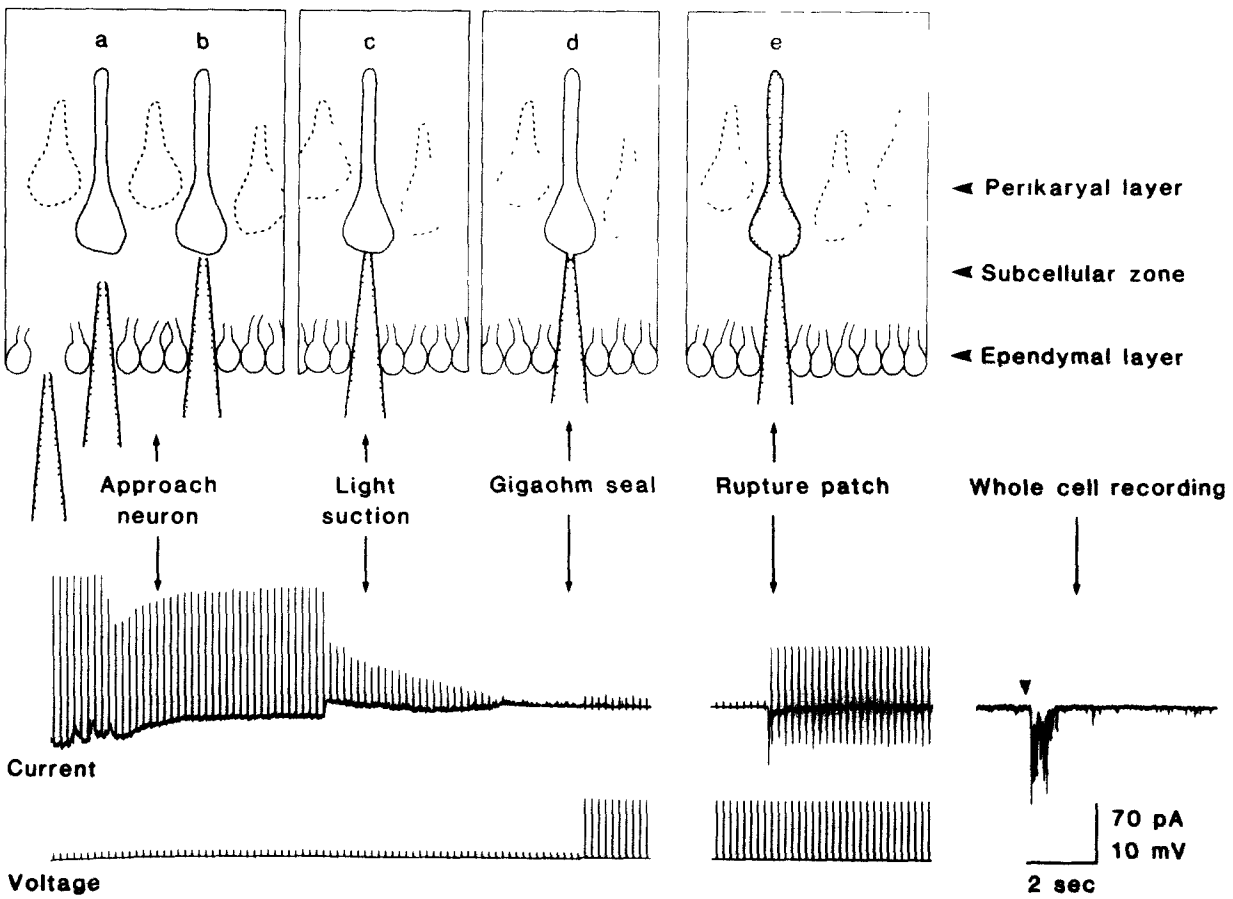

Fig 2. Events in the formation of a whole-cell recording in intact turtle cerebral cortex. The upper panel depicts the electrode (stippled) bypassing the ependymal glial layer, then approaching and sealing onto a neuron, these events are monitored (lower panel) by observing the amplitude of the current produced by small voltage steps applied to the pipette. Decrease in the current amplitude (a,b) reveals a resistance increase as a neuron is approached. Application of light suction (c) results in a further decrease in current amplitude as a gigaohm seal forms (d), more easily measured if the voltage step amplitude is increased. The patch is ruptured by additional suction, and electrical continuity between pipette and cell interior is obtained (e), yielding a whole-cell recording. Stimulation of the optic tract in a turtle hemisphere preparation (▼) produced a barrage of synaptic currents (Vh = −70 mV).

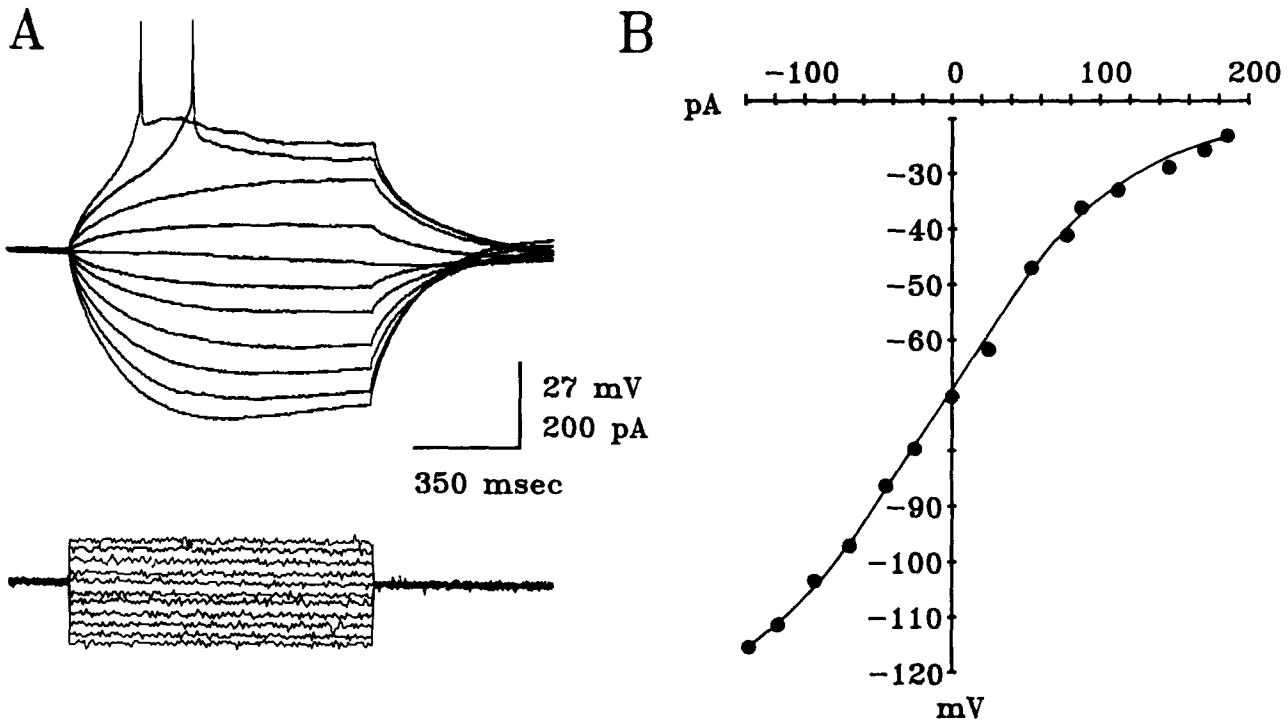

Fig 3 A Voltage responses to current pulse injection in current clamp mode in embryonic (stage 22) neuron in turtle cortex B plot of current-voltage relation for the same cell, illustrating long time constants and high input impedances characteristic of neurons recorded by this method

tained. Neurons had high input impedances compared to those recorded by Connors and Kriegstein (1986) (314 ± 187 MΩ, $n = 23$) and long membrane time constants (tau = 171 ± 73 msec, $n = 23$)

A typical recording in current clamp mode from an embryonic (stage 22) turtle pyramidal neuron is shown in Fig. 3. This cell had a stable resting potential and fired repetitively when depolarized When the membrane was stepped to a series of increasingly depolarized levels in voltage clamp, large transient inward currents appeared; such currents were probably not under voltage control (not shown).

By slowly varying membrane potential in voltage clamp and letting the cell stabilize at each potential, synaptic currents could be studied over a wide voltage range. Thalamic stimulation in a hemisphere preparation produced distinct excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs, Fig. 4), that reversed at -2 and -52 mV, near the equilibrium potentials for cation (0 mV) and Cl^- (-55 mV) conductances respectively The EPSC/IPSC sequence could be easily discerned in voltage-clamp; evoked synaptic potentials in current-clamp from the same cell are shown in Fig 4B for comparison.

Recordings in rat cortex

Recordings were made in rat hippocampal and neocortical slices. With recording solution 1, typical resting potentials in hippocampal pyramidal cells ranged from -46 to -70 mV, and input impedances ranged from 210 to 1600 MΩ. With the cesium containing intracellular solution, input resistances were 2–3 times greater

Fig. 5 shows responses of a layer II-III pyramidal cell in visual cortex of a P43 rat. The EPSC was isolated by blocking $GABA_A$ receptor mediated inhibition with bicuculline (5 μM). Early and late components of the EPSC in disinhibited slices reversed at the same potential (5 mV) but differed in their voltage-dependence (Fig. 5B).

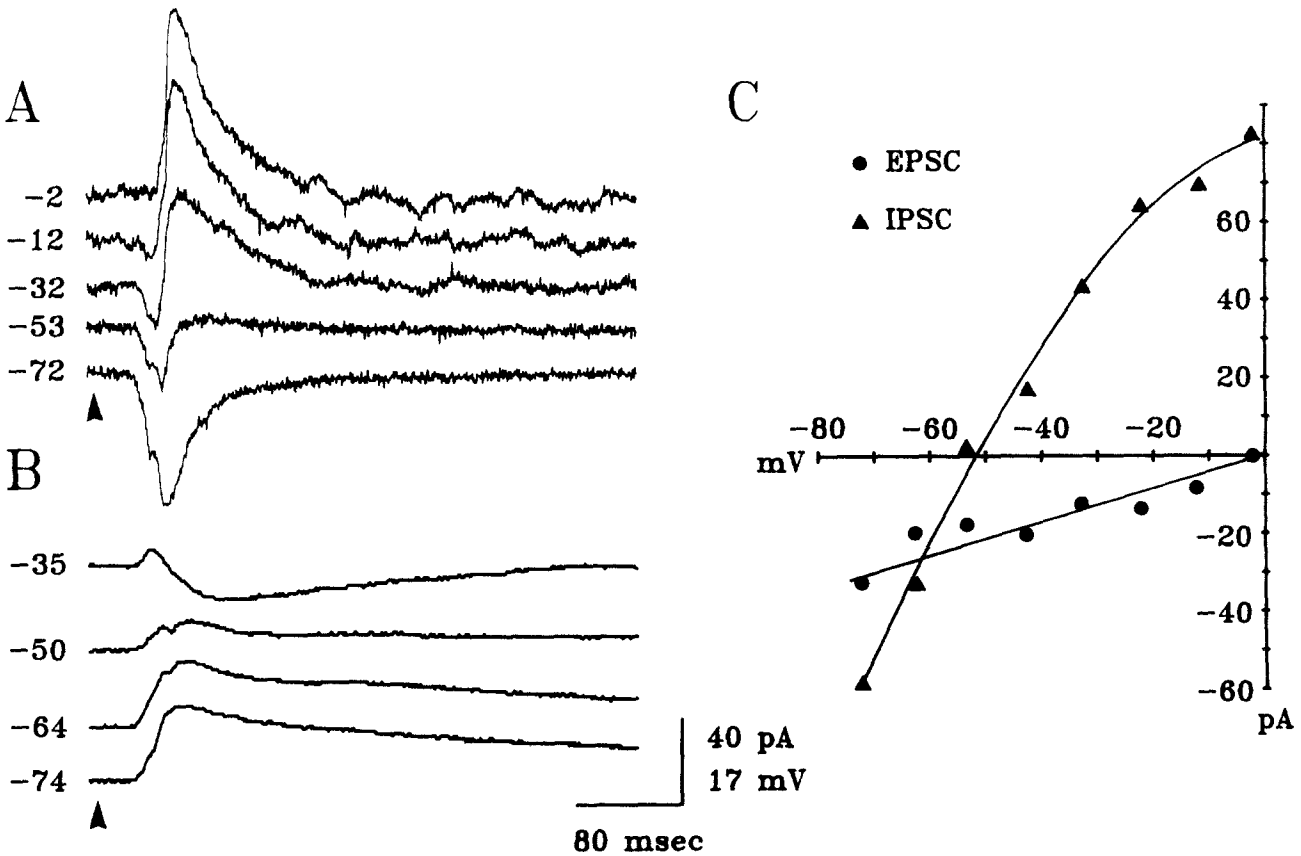

Fig. 4 Synaptic currents evoked by thalamocortical stimulation, recorded in voltage clamp (A) in stage 22 turtle neuron shown in Fig 3, and (B) the synaptic potentials produced by these currents recorded in current clamp C plot of excitatory (EPSC) and inhibitory (IPSC) synaptic currents from (A).

Recordings in noncortical structures

Whole cell recordings were obtained from a variety of turtle brain regions, including the retina, thalamus, optic tectum, basal forebrain, and cerebellum. Brain regions with clear laminar structure (cortex, cerebellum, optic tectum but not retina) were easier to record from than those with nuclear organization (thalamus, basal forebrain). Cortical, cerebellar, and tectal recordings were obtained by approaching the cells from the ventricular surface in intact hemisphere preparations of hatchling turtles.

Anatomical-physiological correlations

To correlate physiological features with morphology, lucifer yellow was included in the pipette solution and injected into cells with hyperpolarizing pulses. LY-filled electrodes exhibited excellent recording properties, allowing routine identification of all recorded cells after each experiment.

Discussion

Our results demonstrate that whole-cell recording techniques can be applied to study synaptic currents and membrane properties in neurons of the turtle and mammalian cerebral cortex. Recordings can be obtained from neurons in a variety of brain regions and from animals ranging in age from embryonic to adult. The techniques described here are easily implemented with a standard intracellular recording apparatus, additionally requiring only a suitable voltage clamp

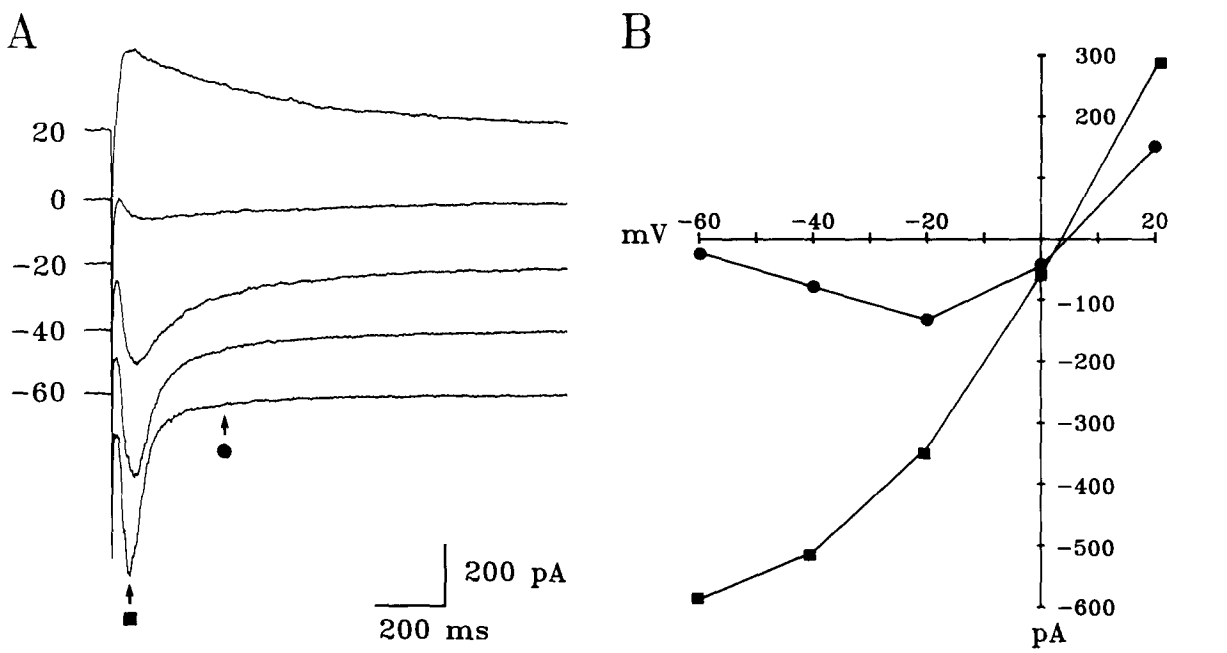

Fig 5 Synaptic currents recorded from a layer II–III neocortical pyramidal neuron in the presence of 5 μM bicuculline A synaptic currents recorded at different membrane potentials B plot of the early and late components of the currents shown in A

amplifier. Application of the fibrin clot technique (Harrison, 1910; Takahashi, 1978) provided excellent tissue stabilization and convenience for recording.

Interpretation of voltage- and current-clamp recordings from intact neurons with long neurites requires an awareness that distal neuronal membranes may not be isopotential with the soma (Rall and Segev 1985; Carnevale and Johnston 1982). However, it has been suggested that the electrical length of neuronal dendrites has been overestimated by current models (Glenn 1988). Moreover, the high input impedances observed with whole cell recording (Coleman and Miller 1989, this study) indicate that neurons are more electrotonically compact than thought from conventional microelectrode recording, thus facilitating the recording of distal events. In the data reported here, the correspondence of synaptic reversal potentials recorded in voltage clamp to the expected equilibrium potentials would suggest that the synaptic events occur at sites isopotential with the soma. Even distal dendritic membrane may be under voltage control since specific activation of thalamocortical synapses in turtle, which are located exclusively on the distal dendrites (Smith et al., 1980), produces synaptic currents that reversed near the expected equilibrium potentials

The methods detailed here offer the advantages of whole cell recording for cells in relatively intact neural structures The low resistance access to the cell interior allows better control of membrane voltage for assessing synaptic events, and the high resistance of the electrode seal gives a better approximation of cell intrinsic membrane properties. The relatively large tip diameters of patch pipettes allow ready exchange of electrode solution for control of the intracellular milieu and allow introduction of kinases and other proteins difficult to inject at known concentration with sharp electrodes (Neher, 1988). Finally, small or immature neurons difficult to record from with conventional microelectrodes can be studied in situ. Whole-cell recording methods should, therefore, prove useful for studies in a variety of neural preparations

Acknowledgements

We would like to thank Dr. John Huguenard for technical advice and John Avilla for technical assistance. We thank Dr. Gerald Crabtree for suggestions on speeding the clotting procedure. Mary Ellen Denison helped with word processing. We appreciate critical comments on the manuscript provided by Drs. Istvan Mody and John Huguenard. This work was supported by N.I.H. grants NS12151, NS00887 and NS21223; M.B. was supported by Stanford M.S.T.P. training grant GM07365.

References

Barnes, S and Werblin, F (1986) Gated currents generate single spike activity in amacrine cells of the tiger salamander retina, Proc Natl. Acad Sci. USA, 83 1509–1512

Carnevale, N T and Johnston, D (1982) Electrophysiological characterization of remote chemical synapses, J Neurophysiol., 47. 606–621

Coleman, P A and Miller, R.F (1989) Measurement of passive membrane parameters with whole-cell recordings from neurons in the intact amphibian retina, J Neurophysiol , 61 218–230.

Connors, B W and Kriegstein, A.R (1986) Cellular physiology of the turtle visual cortex. Distinctive properties of pyramidal and stellate neurons, J. Neurosci , 6(1): 164–177

Connors, B W , Gutnick, M J and Prince, D A (1982) Electrophysiological properties of neocortical neurons in vitro J Neurophysiol , 48 1302-1320

Dingledine, R (Ed) (1984) Brain slices, Plenum Press New York, NY, 442 pp

Glenn, L L (1988) Overestimation of the electrical length of neuron dendrites and synaptic electrotonic attenuation, Neurosci Lett , 91 112–119

Harrison, R G (1910) The outgrowth of the nerve fiber as a mode of protoplasmic movement, J Exp Zool , 9 787–846

Konnerth, A , Edwards, F and Sakmann, B (1988) GABAergic synaptic and single channel currents recorded in rat hippocampal slices, Pflugers Arch , 411 R149

Mori, K , Nowycky, M.C and Shepherd, G M (1981) Electrophysiological analysis of mitral cells in the isolated turtle olfactory bulb, J Physiol. (Lond), 314 281 294

Neher, E (1988) The use of the patch clamp technique to study second messenger-mediated cellular events, Neurosciences, 26 727–734

Sakmann, B and Neher, G (Eds) (1983) Single channel recording, Plenum Press, New York, NY, 503 pp

Smith, L M , Ebner, F F and Colonnier, M (1980) The thalamocortical projection in *Pseudemys* turtles a quantitative electron microscopic study J Comp Neurol , 190 445–461

Taghert, P H , Bastiani, M J , Ho, R K and Goodman, C S (1982) Guidance of pioneer growth cones filopodial contacts and coupling revealed with an antibody to Lucifer Yellow Dev Biol , 94 391–399

Takahashi, T (1978) Intracellular recording from visually identified motoneurons in rat spinal cord slices, Proc R Soc Lond B, 202 417–421.