January 1997

Axopatch-1D
PATCH CLAMP
THEORY AND OPERATION

Part Number 2500-119 Rev E, Printed in U.S.A.

DISCLAIMER
THIS EQUIPMENT IS NOT INTENDED TO BE USED AND SHOULD NOT BE USED IN HUMAN EXPERIMENTATION OR APPLIED TO HUMANS IN ANY WAY.

WARNING
IF THIS EQUIPMENT IS USED IN A MANNER NOT SPECIFIED BY THE MANUFACTURER, THE PROTECTION PROVIDED BY THE EQUIPMENT MAY BE IMPAIRED.

Power-Supply Voltage Selection and Fuse Changing

Supply Voltage
The Axopatch-1D is set at the factory to accept either 115 or 230 V~. The selected supply voltage is marked on the rear panel. The frequency can vary between 50 and 60 Hz. The unit uses 20 Watts of power.

Changing the Fuse
The Axopatch-1D uses a 250 V~, T0.5A, 5 x 20 mm fuse.

In the event of fuse failure, disconnect the power cord.

Before changing the fuse investigate the reason for its failure.

To change the fuse:
1. Disconnect the power cord.
2. Use a screwdriver or a similar device to rotate the fuse holder counterclockwise.
3. Replace the fuse with another fuse of the same rating.
4. Reconnect the power cord.

Basic Equipment Setup and Safety

1. Supply and Earthing Connections: Use the included IEC power cord to connect the instrument to a GROUNDED power receptacle.
2. Mounting: Table or rack.
3. Assembly: Attach the CLAMP and BATH headstages to their labeled connectors on the rear of the instrument.
4. Use: Do not operate this equipment with covers or panels removed.
5. Cleaning: Wipe the headstage connector with a damp cloth to clean salt spills. Avoid spilling liquids on the headstage.

   The Teflon input connector should be kept very clean. Effective cleaning can be done by spraying with alcohol or swabbing carefully with deionized water. If possible, avoid the use of Freon since it is thought to be detrimental to the environment.

Safe Environmental Conditions
1. Indoor use.
2. Mains supply fluctuations: not to exceed ±10% of the nominal voltage.
3. Temperature: between 5 ºC and 40 ºC.
4. Altitude: up to 2000 m
5. This instrument is designed to be used under laboratory conditions. Operate in a clean, dry environment only. Do not operate in a wet or damp environment.

Static Precautions
The headstage can normally be safely handled. However, if you are in a laboratory where static is high (*i.e.*, you hear and feel crackles when you touch things), you should touch a grounded metal object immediately before touching the headstage.

WARNING
Shipping the Axopatch-1D

The Axopatch-1D is a solidly built instrument designed to survive shipping around the world. However, in order to avoid damage during shipping, the Axopatch-1D must be properly packaged.

In general, the best way to package the Axopatch-1D is in the original factory carton. If this is no longer available, we recommend that you carefully wrap the Axopatch-1D in at least three inches (75 mm) of foam or "bubble-pack" sheeting. The wrapped Axopatch-1D should then be placed in a sturdy cardboard carton. Mark the outside of the box with the word FRAGILE and an arrow showing which way is up.

We do not recommend using loose foam pellets to protect the Axopatch-1D. If the carton is dropped by the shipper, there is a good chance that the Axopatch-1D will shift within the loose pellet packaging and be damaged.

If you need to ship your Axopatch-1D to another location, or back to the factory, and you do not have a means to adequately package it, Axon Instruments can ship the proper packaging material to you for a small fee. This may seem like an expense you would like to avoid, but it is inexpensive compared to the cost of repairing an instrument that has sustained shipping damage.

It is your responsibility to package the instrument properly before shipping. If it is not, and it is damaged, the shipper will not honor your claim for compensation.

LIMITE DE RESPONSABILITE
CE MATERIEL N'A PAS ETE CONCU POUR DES EXPERIENCES SUR LES ETRES HUMAINS; ET NE DOIT DONC PAS ETRE UTILISE A CETTE FIN.

ATTENTION
L'EMPLOI DE CE MATERIEL D'UNE MANIERE DIFFERENTE A CELLE SPECIFIEE PAR LE FABRICANT AFFECTERA LE NIVEAU DE PROTECTION FOURNIT PAR L'APPAREIL.

Sélection du voltage et changement du fusible

Voltage d'alimentation

L'Axopatch-1D est réglé à la fabrique pour accepter une alimentation du 115 ou 230 V~. L'alimentation selectée est indiquée sur le panneau arrière. La fréquence peut varier entre 50 et 60 Hz. L'appareil demande 20 Watts.

Changement du fusible

L'Axopatch-1D emploie un fusible de 250 V~, T0.5A, 5 × 20 mm.

En cas de rupture du fusible, débrancher la prise de courant.

Avant de changer le fusible, chercher la raison de la panne.

Pour changer le fusible:
1. Débrancher la prise de courant.
2. A l'aide d'un tournevis ou autre outil de ce genre, faire tourner le support du fusible dans de sens opposé des aiguilles d'une montre.
3. Remplacer le fusible par un fusible de même valeur.
4. Rebrancher la prise de courant.

Installation du matériel et sécurité
1. Branchement: Employer le fil electrique IEC fourni pour brancher l'appareil a une prise de courant comprenant UNE TERRE.
2. Pose: Table ou rack.
3. Montage: Attache la tête de l'amplificateur ("CLAMP headstage") et la tête de l'amplificateur du bain ("BATH headstage") à l'appareil à leurs prises respectives, indiquées sur le panneau arrière.
4. Emploi: Ne pas utiliser ce matériel sans son couvercle et ne pas le couvrir lors de son utilisation.
5. Nettoyage: Essuyer la prise du "headstage" avec un linge humide pour nettoyer les traces de sel. Eviter de renverser des liquides sur le "headstage".
   La prise d'entrée en Téflon doit être maintenue trés propre. Un nettoyage efficace consiste à vaporiser de l'alcool ou á essuyer soigneusement avec de l'eau désionisée. Si possible, éviter l'emploi de Fréon, ce produit étant considéré comme nuisible pour l'environnement.

Conditions à respecter pour un emploi sans danger
1. Emploi à l'intérieur.
2. Fluctuations du réseaux d'alimentation: ne doivent pas dépasser ±10% de la tension nominale.
3. Température: entre 5 °C et 40 °C.
4. Altitude: jusqu'à 2000 m.
5. Cet appareil a été étudié pour l'emploi en laboratoire et il doit être situé dans un environnement sec et propre. Ne pas l'utiliser dans un environnement mouillé ou humide.

Précautions statiques

Le "headstage" peut être maniée sans danger. Cependant, dans un laboratoire avec un niveau élevé d'electricité statique (c'est-à-dire lorsque vous sentez et voyez des décharges électriques), touchez un objet métallique pour une mise à la terre avant de toucher le "headstage".

ATTENTION
Expédition de l'Axopatch-1D

L'Axopatch-1D est un appareil de construction robuste, étudié en vue d'expéditions dans le monde entier. Cependant, l'appareil doit être correctement emballé pour éviter tout domage pendant son transport.

En général, la meilleure façon d'emballer l'Axopatch-1D est de le mettre dans son carton d'origine. Si celui-ci n'est plus disponible, il est recommandé d'envelopper soigneusement l'Axopatch-1D dans au moins trois inches (75 mm) de mousse ou de feuilles d'emballage à bulles. L'Axopatch-1D ainsi protégé devra alors être placé dans un carton solide. Indiquer la mention FRAGILE sur l'extérieur de la boîte ainsi qu'une flèche vers le haut montrant la position verticale.

Il n'est pas recommandé d'employer des boulettes de mousse pour protéger l'Axopatch-1D. En cas de chute de la boîte durant son transport, l'Axopatch-1D pourrait se déplacer à l'intérieur et être endommagé.

Si vous devez expédier l'Axopatch-1D à un autre endroit, ou le renvoyer au fabricant, et si les matériaux d'emballage nécessaires corrects ne sont pas disponibles, ces derniers peuvent être obtenus chez Axon Instruments pour un prix minime. Bien que ceci puisse sembler être une dépense que vous pourriez éviter, elle est cependant insignifiante en comparaison à celle que coûterait la réparation d'un appareil endommagé pendant le transport.

La responsabilité vous incombe de bien emballer l'appareil avant son expédition. Si ceci n'est pas fait, le transporteur ne pourra pas satisfaire vos réclamation de compensation en cas d'avaries.

UNZULÄSSIGE VERWENDUNG
DIESER APPARAT IST NICHT VORGESEHEN, BEI MENSCHLICHEN VERSUCHEN VERWENDET ZU WERDEN UND AUCH NICHT AN MENSCHEN IN IRGENDEINERWEISE ANWENDBAR.

WARNUNG
WEN DIESER APPARAT IN EINER ART UND WEISE ANGEWENDET WIRD, DIE NICHT VOM HERSTELLER SPEZIFISCH ERWÄHNT WIRD, KANN DIE SCHUTZVORRICHTUNG DES APPARATES BEEINTRÄCHTIGT WERDEN.

Spannungswahl für die Stromversorgung und Auswechseln der Sicherung

Netzspannung

Das Axopatch-1D wird bei der Fabrik auf 115 V~ oder 230 V~ eingenstellt. Die entsprechende Netzspannung ist an der Rückseite kennenzeichnet. Die Frequenz kann zwischen 50 und 60 Hz schwanken. Der Gerät braucht 20 Watts.

Auswechseln der Sicherung

Der Axopatch-1D verwendet eine 250V~, T0.5A, 5 × 20 mm Sicherung.

Im Falle des Ausfalls der Sicherung das Netzkabel ausschalten.

Vor dem Auswechseln der Sicherung den Grund für ihren Ausfall untersuchen.

Schritte zum Auswechseln der Sicherung:
1. Das Netzkabel ausschalten.
2. Die Fassung der Sicherung mit einem Schraubenzieher oder einem ähnlichen Werkzeug entgegen dem Uhrzeiger drehen.
3. Die Sicherung mit einer anderen Sicherung mit gleicher Nennleistung ersetzen.
4. Das Netzkabel wieder anschließen.

Grundlegende Hinweise zu Installation und Sicherheit der Ausrüstung
1. Netz- und Erdungsanschlüsse: Das Instrument mit dem beigefügten IEC Netzkabel an einen Erdungsschalter anschließen.
2. Anbringung: Tisch oder Rahmengestell.
3. Montage: Verbinden Sie die Vorverstärker ("CLAMP headstage") und Lösungsvorverstärker ("BATH headstage") zu den entsprechenden beschrifteten Schaltern an der Rückseite des Gerätes.
4. Gebrauch: Dieser Apparat darf nicht mit abgenommenen Abdeckungen oder Platten in Betrieb gesetzt werden.
5. Reinigung: Zur Reinigung von verschüttetem Salz den Vorverstärkeranschluß mit einem feuchten Tuch abwischen. Das Verschütten von Flüssigkeiten auf den "headstage" ist zu vermeiden.

   Der Teflon-Eingangsstecker sollte in sehr sauberem Zustand gehalten werden. Durch Besprühen mit Alkohol oder vorsichtigem Abtupfen mit entionisiertem Wasser ist eine wirksame Reinigung möglich. Die Benutzung von Freon ist nach Möglichkeit zu vermeiden, da diese Substanz als umweltschädigend angesehen wird.

Umweltsichere Betriebsbedingungen
1. Verwendung in Innenräumen.
2. Netzschwankungen: darf nicht ±10% der Nennspannung überschreiten.
3. Temperatur: zwischen 5 °C und 40 °C.
4. Höhe: bis zu 2000 m.
5. Dieses Instrument ist für den Gebrauch unter Laborbedingungen vorgesehen. Nur in sauberer, trockener Umgebung in Betrieb setzen. Nicht in nasser oder feuchter Umgebung in Betrieb setzen.

Schutzmaßnahmen gegen statische Aufladung

Der "headstage" kann normalerweise sicher gehandhabt werden. Falls Sie sich jedoch in einem Labor mit höher statischer Aufladung befinden (*D.h.* Sie hören und fühlen beim Berühren von Objekten ein Knacken), sollten Sie unmittelbar vor dem Berühren der "headstage" ein geerdetes Objekt aus Metall anfassen.

WARNUNG
Versand des Axopatch-1D

Bei dem Axopatch-1D handelt es sich um ein solide gebautes Instrument, das beim weltweiten Versand keinen Schaden nehmen sollte. Um jedoch Versandschäden zu verhindern, muß der Axopatch-1D ordnungsgemäß verpackt werden.

Im allgemeinen läßt sich der Axopatch-1D am besten im Originalkarton des Werks verpacken. Ist dieser nicht mehr vorhanden, empfehlen wir, den Axopatch-1D vorsichtig in mindestens 75 mm starkem Schaumstoff oder Bubblepackungen einzuwickeln. Der so eingewickelte Axopatch-1D sollte dann in einen festen Pappkarton gesetzt werden. Die Außenseite des Kartons ist mit dem Worten ZERBRECHLICH (FRAGILE) und einem Pfeil, der auf die Oberseite des Kartons weist, zu kennzeichnen.

Sollte der Karton vom Spediteur fallengelassen werden, besteht eine gute Möglichkeit, daß der Axopatch-1D innerhalt der losen Schaumstoffkugelverpackung bewegt wird und dadurch beschädigt werden kann.

Wenn Sie den Axopatch-1D an einen anderen Ort oder zurück ans Werk senden müssen und Ihnen kein angemessenes Verpackungsmaterial zur Verfügung stehen, kann Axon Instruments Ihnen das geeignete Verpackungsmaterial gegen eine kleine Gebühr zustellen. Sie mögen dies zwar als unnötige Zusatzkosten betrachten, doch ist dieser Aufwand im Vergleich zu den Reparaturkosten fur ein während des Transports beschädigtes Instrument gering.

Sie sind selbst für das richtige Verpacken des Instruments vor dem Versand verantwortlich. Bei einer nicht ordnungsgemäßen Verpackung, die eine Beschädigung zur Folge hat, wird der Spediteur ihren Schadensersatzanspruch nicht anerkennen.

LÍMITE DE RESPONSABILIDADES
ESTE EQUIPO NO ESTÁ DISEÑADO PARA USO EN HUMANOS Y NO DEBE USARSE PARA EXPERIMENTACIÓN O APLICACIÓN EN SERES HUMANOS BAJO NINGUNA CIRCUNSTANCIA.

ADVERTENCIA
SI ESTE EQUIPO SE USA DE MANERA NO ESPECIFICADA POR EL FABRICANTE SE PODRÍA PERDER LA PROTECCIÓN PROVISTA POR EL EQUIPO.

Selección del suministro de corriente y cambio de fusibles

Voltaje de entrada
El Axopatch-1D está ajustado en la fábrica para un voltaje de 115 o de 230 V~. El voltaje escogido está marcado en el tablero posterior. La frecuencia puede variar entre 50 y 60 Hz. La unidad utiliza 20 vatios.

Cambio de fusible
El Axopatch-1D utiliza un fusible de 250 V~, T0.5A, 5 × 20 mm.
En el caso de que un fusible falle, desconecte el cordón eléctrico.
Antes de cambiar el fusible investigue la causa de la falla.
Para cambiar el fusible:

1. Desconecte el cordón eléctrico.
2. Use un destornillador o un dispositivo similar para girar el portafusibles en sentido contrario al de las manecillas del reloj.
3. Reemplace el fusible existente con otro de la misma capacidad.
4. Conecte nuevamente el cordón eléctrico.

Instalación básica y seguridad del equipo

1. Suministro de corriente y conexión a tierra: Use el cordón eléctrico IEC incluido para conectar el instrumento a una toma de corriente CON CONEXIÓN A TIERRA.
2. Montaje: Sobre una mesa o en un estante.
3. Ensamblaje: Conecte el cabezal del instrumento ("CLAMP headstage") y el de la solución ("BATH headstage") en los conectores respectivos, marcados en el tablero posterior del aparato.
4. Uso: No utilice este equipo sin las cubiertas o paneles.
5. Limpieza: Limpie el conector del "headstage" con un paño húmedo a fin de quitar los derrames de sales. Evite derramar líquidos sobre el "headstage".
   El conector de entrada fabricado de Teflon debe mantenerse muy limpio. Puede hacerse una limpieza efectiva rociando con alcohol o con un algodón humedecido con agua desionizada. En la medida de lo posible evite el uso del gas freón, puesto que es dañino para el medio ambiente.

Condiciones de seguridad ambiental

1. Para uso interior.
2. Fluctuaciones eléctricas en la fuente de suministro: no deben exceder ±10% del voltaje nominal.
3. Temperatura: entre 5 °C y 40 °C.
4. Altitud: hasta 2.000 m
5. Este instrumento está diseñado para ser usado en condiciones de laboratorio. Debe operarse únicamente en un ambiente limpio y seco. No lo use en un ambiente húmedo ni mojado.

Precauciones contra la estática
El "headstage" puede manejarse con seguridad, bajo condiciones normales. Sinembargo, si usted se encuentra en un laboratorio donde la estática es alta (por ejemplo, si escucha y percibe chispas cuando toca los objetos), usted debería tocar inmediatamente un objeto metálico que esté en contacto con tierra, antes de tocar el "headstage".

ADVERTENCIA
Envío del Axopatch-1D
El Axopatch-1D es un instrumento de construcción sólida, diseñado para soportar el transporte a cualquier parte del mundo. Sinembargo, para evitar los daños que pudieran ocurrir durante su envío, el Axopatch-1D debe empacarse adecuadamente.

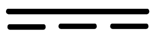
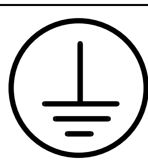
En general, la mejor manera de empacar el Axopatch-1D es en la caja original de fábrica. Si ésta ya no se encuentra disponible, le recomendamos que envuelva cuidadosamente el Axopatch-1D en una funda o sábana de espuma o de "empaque de burbujas" con un espesor mínimo de 3 pulgadas (75 mm). El Axopatch-1D, envuelto así, deberá colocarse en una caja de cartón resistente. Marque el exterior de la caja con la palabra FRÁGIL y una flecha que indique la posición hacia arriba.

No recomendamos el uso de bolitas de espuma sueltas para proteger el Axopatch-1D. Si la caja se cae accidentalmente durante el transporte, es muy probable que el Axopatch-1D se desplace dentro del contenedor con las bolitas de espuma sueltas y se dañe.

Si necesita enviar su Axopatch-1D a otra localidad, o de regreso a la fábrica, y no posee el empaque adecuado, Axon Instruments puede enviarle el material necesario por un cargo mínimo. Esto podría parecerle un gasto superfluo que preferiría evitar, pero es económico comparado con lo que costaría la reparación de un instrumento que ha sufrido daños durante el envío.

Es su responsabilidad empacar el instrumento adecuadamente antes de enviarlo. Si no lo hace así y resulta dañado, el transportista no será responsable ni aceptará su reclamo de indemnización.

Explanation of symbols
Explication des symboles
Erklärung der verwendeten symbole
Explicación de símbolos

Symbol Symbole Symbol Símbolo	Description Description Beschreibung Descripción
	Direct current Courant continu Gleichstrom Corriente continua
~	Alternating current Courant alternatif Wechselstrom Corriente alterna
|	On (Supply) Allumé (alimentation) An (Netz) Encendido (suministro)
O	Off (Supply) Éteint (alimentation) Aus (Netz) Apagado (suministro)
	On (Supply) Allumé (alimentation) An (Netz) Encendido (suministro)
	Off (Supply) Éteint (alimentation) Aus (Netz) Apagado (suministro)
	Protective conductor terminal Borne du conducteur de protection Schutzleiterpol Terminal de conductor protector

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VERIFICATION

THIS INSTRUMENT IS EXTENSIVELY TESTED AND THOROUGHLY CALIBRATED BEFORE LEAVING THE FACTORY. NEVERTHELESS, RESEARCHERS SHOULD INDEPENDENTLY VERIFY THE BASIC ACCURACY OF THE CONTROLS USING RESISTOR/CAPACITOR MODELS OF THEIR ELECTRODES AND CELL MEMBRANES.

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Front and Rear Panels of the Axopatch-1D

INTRODUCTION

The Axopatch-1D is a tight-seal patch clamp for single-channel and whole-cell voltage clamping. It is the fourth and most powerful version of the Axopatch series. Each version introduced enhanced flexibility and performance, thus maintaining our goal to provide the most sophisticated and advanced instruments.

Patch clamping is an extremely powerful technique enabling direct observations to be made on the behavior of single molecular units. It is the biological equivalent of high-energy particle colliders used to investigate atomic interactions. Yet for all its power, patch clamping is a relatively simple technique. Extracting the most from the technique, though, is a demanding task requiring meticulous attention to detail in the design of the equipment and experimental techniques used.

The Axopatch-1D maximizes the useful information that can be measured. It does this by using ultralow-noise semiconductor technology for the headstage and high-speed low-noise circuitry throughout. This fundamental performance advantage is complemented by features and layout designed to expand the range of experiments that can be performed.

To further assist you in your experiments we have endeavored to write this manual not only as a succinct guide to the operation of the Axopatch-1D but also as a guide to experimental techniques that we have found useful during several years of patch clamping. A modest amount of theory has been included to round out and provide the basis for the practical explanations.

We hope you find this manual to be a useful laboratory companion. We aim to revise it regularly and look forward to receiving any suggestions you might have for improvement.

Axon Instruments, Inc.

NOTE

The Axopatch-1D is supplied with the U-type headstage(s). This type of headstage only connects with “U” (universal) type adapters, pipette holders and model cells. The U-type design offers several advantages and these are detailed in the section of the manual entitled *Holders*. The non-U-type headstages can still be used with the Axopatch-1D, but these headstages offer less advantages than their U-type counterpart.

Because Axon Instruments sells replacement pipette holders, adapters and model cells in two varieties, the U-type and the non-U-type, please specify the complete name of the product.

FEATURES

The Axopatch-1D is a tight-seal patch-clamping system for single-channel and whole-cell recording. It features outstandingly low noise and high speed.

The versatile series-resistance and capacitance-compensation features are powerful yet simple to use. Built-in command generators, a variable-cutoff lowpass filter, leak subtraction, DC offset removal, variable output gain and an RMS noise monitor simplify your experimental setup. A digital tracking junction null, seal-test commands, zap and an audio monitor assist in the initial stages of an experiment.

The Axopatch-1D works in current-clamp mode as well as the more usual voltage-clamp mode. The feedback resistor may be remotely switched to enable single-channel and whole-cell currents to be recorded in the same cell.

In the Axopatch-1D, ease of use and engineering excellence are equally well implemented. The Axopatch-1D was designed in a close collaboration between Drs. R.A. Levis, J.L. Rae and R.S. Eisenberg of Rush Medical College, and Dr. A. Finkel of Axon Instruments, Inc.

Headstage and Electrode Holder

The **CV-4 headstage** normally supplied with the Axopatch-1D use advanced technology. Critical components are assembled using **hybrid** techniques. The hybrid package is **hermetically sealed** to minimize the likelihood that the feedback resistor characteristics will change with time. The detailed design of the headstage circuit is based on extensive theoretical analysis and laboratory experience.[1] Special techniques are used to ensure **ultralow noise** while maintaining **wide bandwidth**. The **electrode holder** has been custom constructed for low noise.

Three versions of the CV-4 headstage are available. Any CV-4 headstage can be specified at the time of ordering an Axopatch-1D without affecting the price.

1. CV-4-1/100U. This headstage has 500 MΩ (ß = 1) and 50 GΩ (ß = 100) feedback resistors. This is the standard headstage that is automatically supplied if an alternate headstage is not specified. This headstage is appropriate for single-channel recording from patches and for whole-cell recording in cells which require a clamp current of up to 20 nA.

2. CV-4-0.1/100U. This headstage has 50 MΩ (ß = 0.1) and 50 GΩ (ß =100) feedback resistors; it is appropriate for single-channel recording and for whole-cell recording in cells requiring up to 200 nA of current. Series resistance compensation with this headstage is more straight-forward because whole-cell capacitance compensation is disabled; all of the charging current is passed through the 50 MΩ resistor.

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[1] Rae, J.L. and Levis, R.A. (1984). Molecular Physiology, 6:115-162.

3. CV-4B-0.1/100U. This headstage has 50 MΩ and 50 GΩ feedback resistors. It is ideally suited for single-channel recording from artificial bilayer membranes. It differs from the CV-4-0.1/100U headstage in two important ways:

   a. The headstage is guaranteed to be stable for total electrode and bilayer capacitances up to 300 pF. To achieve this, the bandwidth for single channel recording is restricted to about 6 kHz.

   b. The feedback resistor can be remotely switched by a logic-level pulse. To study voltage-activated channels in a bilayer using the CV-4B-0.1/100U headstage, the headstage is initially put into the ß = 100 mode using the front-panel switch. A few milliseconds before the voltage step command, the experimenter supplies a logic-level pulse that switches the headstage into the ß = 0.1 mode. A few milliseconds after the step command, the membrane will have rapidly charged to the new voltage and the headstage can be returned to the ß = 100 mode for recording the single-channel currents.

Capacitance Compensation

Three stages of capacitance compensation are provided. **Fast** and **Slow** compensation are used to charge the pipette capacitance. **Whole-cell** compensation is used to charge the membrane capacitance. The particular whole-cell compensation settings used uniquely determine the magnitude of the series resistance and the cell capacitance.

Series Resistance Compensation

Once the whole-cell compensation is set, a % COMPENSATION control can be used to compensate for the value of the series resistance. A LAG control cuts the high-frequency response to improve stability when large percentage compensations are used.

Junction Null

The potential required to null the junction potentials between the holder, solution and membrane can be set **manually** or **automatically.** In the AUTO TRACK mode the nulling circuit continuously tracks the junction potential and maintains the pipette current at zero. On switching to HOLD the value of the nulling potential is sampled digitally and held indefinitely. An indicator LED slowly flashes to warn you that TRACK or HOLD is in use.

Mode

V-CLAMP (voltage clamp) mode is used to control the pipette potential. This is the conventional method for patch clamping and the primary operational mode for the Axopatch-1D.

I-CLAMP (current clamp) mode is used to control the pipette current. In this mode the series-resistance controls act like the bridge balance on a microelectrode amplifier. The fast and slow capacitance compensation controls act like a multivariable capacitance neutralization.

I = 0 mode sets the pipette current to zero. This allows the opportunity to reset command potentials before switching from V-CLAMP to I-CLAMP.

PATCH-1U Model Cell

The model cell is an invaluable aid when learning to use the Axopatch-1D and while setting up or reconfiguring an experiment. The model cell can emulate three experimental conditions. **Bath** mode is equivalent to touching the electrode to the bathing solution. **Cell** mode emulates a whole-cell recording configuration. **Patch** mode emulates a gigohm seal. The design of the model cell minimizes extraneous noise so that using the model cell yields realistic current records.

Command Potentials

Several voltage commands can be generated internally.

The **Step Command** generator uses a digital-to-analog converter set by a thumbwheel switch. Thus a high degree of precision and repeatability is achieved. Timing is either set externally or by an internal oscillator.

The **Speed Test** generator injects current into the headstage input via the compensation capacitor. This is useful for checking the frequency response of the system. Either a current step at the internal oscillator frequency or an externally generated waveform can be used.

R_{SEAL} Test supplies small step-voltage commands useful for monitoring seal formation. **External** voltage commands can also be used, with the sensitivity conveniently set at the front panel. The **Holding Potential** is easily set on a ten-turn dial.

Indicator LEDs light up to remind you whenever an internal command is activated

Zap

The conventional technique for rupturing a membrane patch to go to whole-cell recording is to apply a pulse of suction. Sometimes this technique damages the cell. **Zap** provides an alternative method. It applies a pulse of voltage across the patch that ruptures the patch, presumably by causing dielectric breakdown. A timing circuit lets you find a Zap duration that is most likely to achieve the desired result without damaging the seal.

Audio Monitor

In order to watch the preparation without interruption, an audio monitor allows seal formation to be followed aurally. The pitch of the loudspeaker is determined by the value of the selected signal (pipette current or voltage). Earphones can be optionally used to avoid disturbing others in the same room and to prevent microphonic feedback into the electrode.

RMS Noise Monitor

A useful measure of the quality of the electrode and seal is the RMS current noise. It is continuously measured in a 5 kHz bandwidth and displayed on a dedicated digital meter.

Main Meter

A 3½-digit voltmeter is used to display the tracking potential, the membrane potential, the bath potential or the electrode current. The electrode current is conveniently displayed directly in pA or nA.

Outputs

The **Scaled Output** is the membrane potential (V_m) or current (I) with scaling and filtering provided by an 8-position GAIN switch and a 4-pole 12-position lowpass Bessel filter**.** Proportional voltage outputs are provided to transmit the gain and frequency settings to a computer.

Resistive **Leak** currents can be simply subtracted using a front-panel potentiometer; more complex passive currents can be subtracted by applying the waveform to be subtracted to the Leak Subtract input.

The **Auto Output Zero** operates to zero the DC value of the selected signal on the Scaled Output. This zeroing circuit can be reset from the front panel or by an external logic pulse. If this external pulse is synchronized with the oscilloscope trigger, nondistorting AC coupling is achieved. Since the Auto Output Zero circuit is implemented digitally, there is no drift after a sample is taken.

So as not to crowd the front-panel controls, all inputs and outputs are on the rear panel with only the signals most likely to be connected to an oscilloscope being repeated on the front panel. Internally or externally generated **calibration** pulses can be added to the current and voltage outputs. Selected signals are displayed on a central **digital main meter.**

General

A BH-1 ultralow-noise headstage can be used with an extracellular electrode to record the **bath potential**. This potential is used to compensate for potential shifts caused by changing the bath solution or temperature.

A specially constructed **low-radiation transformer** eliminates the source of line-frequency noise (hum). The incoming line voltage is filtered to remove radio-frequency interference (RFI).

Strong emphasis has been placed on quality. Precision ten-turn potentiometers, reliable switches and gold plated connectors are used throughout. Ultralow-drift low-noise operational amplifiers are used in all critical positions and ICs are socketed for easy maintenance. Detailed operator's and service manuals are provided.

FUNCTIONAL CHECKOUT

When you receive the Axopatch-1D you should first run a functional checkout to ensure the proper functioning of the instrument. All units are thoroughly tested in the factory before shipping. If you observe any damage caused by shipping or if you encounter problems with the functional checkout, please call the factory.

For the initial checkout, the Axopatch-1D should be situated on a benchtop away from other equipment. Do not install in a rack until the checkout is complete. Make sure that the POWER is OFF.

Initially, the only connections to the Axopatch-1D should be: a) The power cable, b) The headstage.

Plug the CV-4 boost box into the indicated space on the rear panel.

1. The headstage. Make no connections to the white input socket. Shield the probe end by wrapping it in grounded aluminum foil or by placing it into a Faraday cage. (Ground is available from the gold-plated 2 mm socket at the rear of the probe, from the yellow 4 mm socket on the rear-panel of the main unit, or from the BNC shields.) Plug the boost box into the indicated space on the rear panel. This is a physical (not an electrical) connection used to store the boost box out of the way. Take care to prevent static discharge near the headstage input connector.

2. A BNC cable from the SCALED OUTPUT connector to the vertical input of your oscilloscope. For now, use the BNC connector on the front panel.

3. A BNC cable from the COMBINED OSCILLOSCOPE TRIGGER output to the EXT. TRIGGER input of your oscilloscope.

All Axopatch-1D controls should be set as follows:

Manual Junction Null: About 5 turns from end
Auto Junction Null: OFF
Series Resistance Comp. % Compensation: OFF
Series Resistance Comp. Lag: Full counterclockwise
Step Command: 000.0, OFF button depressed
Speed Test: OFF
Oscillator Freq.: 100 Hz
R_{SEAL} Test: OFF
Ext. Command Sensitivity: OFF
Holding Potential: Counterclockwise, OFF
Zap: 0.1 ms
Main Meter: I button depressed
Capacitance Compensation: All full counterclockwise
Audio monitor: OFF
Output Cal.: OFF
Mode: V-CLAMP
Headstage gain ß: 1/0.1
RMS Noise Display: I (pA,nA)
Gain (α): 1
Output Select: I
Leak Subtraction: ∞
-3 dB Freq.: 2 kHz
Active/Bypass: Active
Auto Output Zero: OFF

Set your oscilloscope to 1 V/div, 5 ms/div. In the Command Potential section, switch SPEED TEST to the OSC. position. A rectangular waveform of approximately 1 volt in amplitude should appear. If the waveform is not stationary, check that your oscilloscope is set for external triggering and adjust the trigger level. The waveform should be high for about 1/3 of each period. The 1/3 ON, 2/3 OFF waveform enables you to easily distinguish the ON and OFF parts of the cycle.

Test the GAIN (α) control. Test the filter. Note that the -3 dB Frequency control sets the bandwidth; the ACTIVE/BYPASS switch switches the filter in and out.

Move the BNC cable from the SCALED OUTPUT connector to the I output. Note that the GAIN and FREQUENCY controls have no effect. All of the controls in the Outputs section **only** affect the SCALED OUTPUT. Return the BNC cable to the SCALED OUTPUT connector. Set α = 1 and bandwidth = 2 kHz.

Change the HEADSTAGE GAIN (ß) to 100. The rectangular waveform should now be approximately 10 volts in amplitude.

Switch SPEED TEST to the OFF position. The current reading on the main meter should be near zero. The reading on the RMS NOISE meter should be less than 0.15 pA RMS. If not, you are probably picking up line-frequency hum due to inadequate shielding of the probe. Notice that as you change ß to various values the decimal points on the meters move so that they always read correctly. For ß = 0.1 the meter readings are in nA; otherwise, the meter readings are in pA.

GETTING UP AND RUNNING - A TUTORIAL

This chapter attempts to ease you into the use and enjoyment of your Axopatch-1D. Please do not be intimidated by the large number of controls. These have been carefully grouped for clarity. Many of them can be switched off and ignored until you become more familiar with the instrument and patch clamping.

Operation of the Axopatch-1D will be described here in the context of a real experiment. If you experience any difficulties at all, we highly recommend that you work through these procedures using the PATCH-1U model cell supplied with the unit and illustrated in the *Model Cell* section in the **REFERENCE GUIDE**.

You will frequently come across the term "ß", which refers to the headstage gain. ß is directly related to the feedback resistor (R_f) in the headstage:

$R_f = 50$ GΩ; ß = 100
$R_f = 500$ MΩ; ß = 1
$R_f = 50$ MΩ; ß = 0.1

Certain controls and outputs are scaled by ß. To simplify the mental arithmetic involved, ß was chosen to be a power of 10.

Note that your CV-4 headstage only contains 2 of the 3 possible feedback resistors. Typically you will have a headstage with ß = 1 and ß = 100. ß = 0.1 can be ordered if you need to pass very large currents (up to 200 nA).

You can select the headstage gain using the HEADSTAGE GAIN (ß) switch. In the "100" position, the headstage gain is 100 on all headstages. In the "1/0.1" position, the headstage gain depends on the particular headstage that you are using. Most commonly the gain will be 1 because you will be using a CV-4-1/100U headstage. If you are using an 0.1/100U headstage, the gain will be 0.1.

Startup Procedure (10 steps to peace of mind)

For the initial checkout, the Axopatch-1D should be situated on a benchtop away from other equipment. Do not install in a rack until the checkout is complete. Make sure that the POWER is OFF.

1. Initially, the only connections to the Axopatch-1D should be:
   a) The power cable.
   b) The headstage.

Plug the CV-4 boost box into the indicated space on the rear panel. This is a physical (not an electrical) connection used to store the boost box out of the way. Take care to prevent static discharge near the headstage input connector.

2. All Axopatch-1D controls should be set as follows:

Manual Junction Null: About 5 turns from end
Auto Junction Null: OFF
Series Resistance Comp. % Compensation: OFF
Series Resistance Comp. Lag: Full counterclockwise
Step Command: 000.0, OFF button depressed
Speed Test: OFF
Oscillator Freq.: 100 Hz
R_{SEAL} Test: OFF
Ext. Command Sensitivity: OFF
Holding Potential: Counterclockwise, OFF
Zap: 0.1 ms
Main Meter: I button depressed
Capacitance Compensation: All full counterclockwise
Audio monitor: OFF
Output Cal.: OFF
Mode: V-CLAMP
Headstage gain ß: 1/0.1
RMS Noise Display: I (pA,nA)
Gain (α): 1
Output Select: I
Leak Subtraction: ∞
-3 dB Freq.: 2 kHz
Active/Bypass: Active
Auto Output Zero: OFF

Test the noise level:

3. Shield the headstage with a large sheet of aluminum foil. Wrap the foil loosely and completely around the headstage. Leave room at the headstage input for the model cell in the next test. Connect the foil shield to the ground input of the headstage (gold-plated 2 mm socket at the rear of the probe) using a clip-lead. The easiest way to do this is to connect the clip-lead directly to the foil and to the 2 mm pin inserted into the headstage ground socket. (Ground is available from the gold-plated 2 mm socket at the rear of the probe, from the yellow 4 mm socket on the rear-panel of the main unit, or from the BNC shields.)

4. Turn the POWER ON. Note the reading on the RMS NOISE display for the two headstage gain values (toggle the HEADSTAGE GAIN switch to ß = 100 and either ß = 1 or ß = 0.1). The expected values under optimal conditions for the CV-4 are:

ß = 100	<0.15 pA
ß = 1	<0.6 pA
ß = 0.1	<0.002 nA

If the observed values are more than twice the expected values or if the meter is blanked due to exceeding its range, then check to see that the foil shield is correctly grounded and that all controls are in the positions noted in 2 above.

Test each ß gain:

5. Remove the foil shield temporarily. Connect the black ground lead (2 mm pin at each end) to the model cell ground (2 mm socket at central position) then connect this lead to the ground input of the headstage.

6. Connect the model cell BATH connection to the headstage input. Insert the Teflon collet of the model cell into the collar of the headstage (the fit will be snug). Make sure that the collet is inserted all the way such that the connector pin is fully inserted into the headstage input jack.

7. Shield the headstage and model cell with the foil. Reconnect the foil shield to ground.

8. Place the HEADSTAGE GAIN switch in the ß = 1/0.1 position. Zero the panel meter using the JUNCTION NULL MANUAL knob. If the meter cannot be zeroed or constantly drifts, then the foil shield is probably not correctly connected to ground.

9. Enter 100.0 on the STEP COMMAND thumbwheel switch and press the CONT. button.

   This clamps 100 mV across the 10 MΩ resistor in the model cell bath position. The main meter should read the correct current (10 nA) within error limits (2% for the meter, 1% for the resistor and 1% for the step command generator plus errors for your zeroing job, etc.).

10. Turn the STEP COMMAND OFF. Place the HEADSTAGE GAIN switch in the ß = 100 position. Zero the panel meter again using the JUNCTION NULL MANUAL knob. Note: the Junction Null will be very sensitive for the higher gain -- just try to get close to zero. If the main meter goes off-scale during zeroing, switch to ß = 1, zero at that setting, then switch back to ß = 100.

    Enter 1.0 on the STEP COMMAND thumbwheel switch. Press the CONT. button below the thumbwheel switch to repeat the test in 9 for ß = 100. This clamps 1.0 mV across the 10 MΩ resistor in the model cell bath position. The main meter should read 100 pA (noise at the higher ß can make this value fluctuate a bit). Note that the main meter automatically adjusts to pA for ß = 100.

Functional Checkout

Make sure that all controls are in the startup position (from 2 above). Initially, the only connections to the Axopatch-1D should be:

1. The headstage. Make no connections to the white input socket. Shield the probe end by wrapping it in grounded aluminum foil or by placing it into a Faraday cage. (Ground is available from the gold-plated 2 mm socket at the rear of the probe, from the yellow 4 mm socket on the rear-panel of the main unit, or from the BNC shields.) Plug the boost box into the indicated space on the rear panel.

2. A BNC cable from the SCALED OUTPUT connector to the vertical input of your oscilloscope. For now, use the BNC connector on the front panel.

3. A BNC cable from the COMBINED OSCILLOSCOPE TRIGGER output to the EXT. TRIGGER input of your oscilloscope.

Set your oscilloscope to 1 V/div, 5 ms/div. In the Command Potential section, switch SPEED TEST to the OSC. position. A rectangular waveform of approximately 1 volt in amplitude should appear. If the waveform is not stationary, check that your oscilloscope is set for external triggering and adjust the trigger level. The waveform should be high for about 1/3 of each period. The 1/3 ON, 2/3 OFF waveform enables you to easily distinguish the ON and OFF parts of the cycle.

Practice using the GAIN (α) control. Practice using the filter. Note that the -3 dB FREQ. control sets the bandwidth. The ACTIVE/BYPASS switch switches the filter in and out. When the ACTIVE/BYPASS switch is in the Active position the filter operates as indicated by the bandwidth setting. When the ACTIVE/BYPASS switch is in the BYPASS position the filter is bypassed, the frequency setting is ignored, and the signal is transferred at full bandwidth.

Move the BNC cable from the SCALED OUTPUT connector to the I output. Note that the Gain and Frequency controls have no effect. All of the controls in the Outputs section **only** affect the Scaled Output. Return the BNC cable to the SCALED OUTPUT connector. Set $\alpha = 1$ and bandwidth = 2 kHz.

Change the HEADSTAGE GAIN (ß) to 100. The rectangular waveform should now be approximately 10 volts in amplitude.

Switch SPEED TEST to the OFF position. The current reading on the main meter should be near zero. The reading on the RMS NOISE meter should be less than 0.15 pA RMS. If not, you are probably picking up line-frequency hum due to inadequate shielding of the probe. Notice that as you change ß to various values the decimal points on the meters move so that they always read correctly. For ß = 1 and ß = 0.1 the meter readings are in nA; otherwise the meter readings are in pA.

Using the Pipette Holder

The HL-U holder, supplied with the Axopatch-1D accepts pipettes with an outer diameter of 1.0 to 1.7 mm.

To minimize noise, it is important to keep the holder clean and dry. Back fill the pipette with a small amount of solution. Loosen the end of the holder (from which the wire protrudes) by about 1 or 2 turns to relieve the pressure on the cone washer inside. Slip the wire (or pellet) into the pipette and slide the pipette up into the holder until it seats. Tighten the holder gently so that the pipette is held firmly in place. Attach some tubing to the stainless-steel tube so that you can apply suction. If all has gone well, only a few millimeters of the silver wire (or pellet) will be immersed.

Making a Seal

Attach an electrode and touch it to the bath solution (alternatively, remove the holder and firmly plug the BATH end of the model cell into the headstage. Remember to connect the ground).

Use ß = 100. The Scaled Output will probably be saturated at plus or minus 13 volts. Use the MANUAL JUNCTION NULL to re-zero the trace. This control compensates for the electrode and holder junction potentials. Set R_{SEAL} TEST at 0.2 mV OSC. A rectangular waveform should appear on your oscilloscope. The amplitude will depend inversely on your electrode resistance. Change the oscillator frequency from 100 Hz to 10 Hz or 1 Hz; 100 Hz gives the most stable oscilloscope trace.

Note that the internal oscillator is only accurate to about 10%. It is provided as an aid to set-up and seal formation; it is not meant to be your primary timing source for experiments.

In the Auto Junction Null section, select the TRACK position. The current waveform will now droop slightly, although at 100 Hz this may not be obvious. The Track circuit slowly adjusts the junction null to keep the electrode current equal to zero. This is useful during seal formation to keep the current trace on your oscilloscope despite transient changes in the pipette offset as the tip presses against the membrane. Move the MANUAL JUNCTION NULL control one turn in either direction. Instead of the current trace moving towards saturation, it is kept centered on the oscilloscope screen by the action of the Track circuit. Track must be disabled (by selecting HOLD or OFF) after the seal is formed to prevent distortion of the current recordings.

Switch ON the AUDIO MONITOR. You will hear a tone modulated by the R_{SEAL} TEST pulses. As you press the electrode against the membrane and apply suction, or when you select the Patch position on the model cell, you will get three indications of the formation of a seal:

1. The amplitude of the test pulses will diminish and disappear into the background.
2. The peak-to-peak noise will become much smaller.
3. The tone from the Audio Monitor will become constant.

You can now switch OFF the AUDIO MONITOR. There are several procedures you can use to switch off Track. The simplest, and the one we will use for now, is just to switch to the HOLD position.

Once you have a seal, or sometimes during seal formation, you will want to know what the seal resistance is. You can always calculate it using Ohm's Law because you know the magnitude of the command and you are observing the magnitude of the current on your oscilloscope. Alternatively, an easy trick can be used with the Axopatch to get the seal resistance without requiring any mental arithmetic.

Use ß = 100. Switch OFF R_{SEAL} TEST. Set your oscilloscope for 100 mV/div, corresponding to 1 pA/div. Press the OSC. button under the Step Command thumbwheel switch. Increase the STEP COMMAND in 1 mV increments until you see a 1 pA peak-to-peak waveform on your oscilloscope. Set the filter -3 dB FREQ. to about 200 Hz if you are having trouble seeing the current waveform. The reading on the thumbwheel switch corresponds to the electrode resistance in GΩ. If you are using the model cell in the Patch position, you should be using around 10 mV on the STEP COMMAND switch. After you have made this measurement, switch off the STEP COMMAND and return the filter -3 dB FREQ. to 2 kHz.

Changing the Membrane Potential

The HOLDING POTENTIAL control is used to impose DC shifts on the membrane. As you change the Holding Potential you will see corresponding changes in the current.

Select V_m on the main meter. This will show you the membrane potential. Note that for the cell-attached patch the initial clamped membrane potential is zero because the potential of the inside of the cell is unknown.

The STEP COMMAND switch can be used to change the membrane potential in several ways. First, select CONT. Now the value on the thumbwheel switch will be continuously applied to the membrane. Follow these changes on your oscilloscope by setting OUTPUT SELECT = V_m. Second, select OSC. The value on the thumbwheel switch is gated by the internal oscillator so that it is applied for about 1/3 of each period and unapplied for 2/3. Third, select GATE. Nothing will happen until you connect a logic-level gating pulse from an external pulse generator.

To do so, take the output of your pulse generator and connect it to the STEP GATE input on the rear panel. Take the trigger output of your pulse generator and connect it to the EXT. OSCILLOSCOPE TRIGGER input. Now the COMBINED OSCILLOSCOPE TRIGGER output will contain either the trigger from the internal oscillator of the Axopatch-1D, or the trigger from your pulse generator, depending on which of them is on. This makes it easy for you to trigger your oscilloscope irrespective of the command source.

Adjust the output of your pulse generator so that it swings at least from 0 V to +5 V, or at most from -15 V to +15 V. Whenever the pulse generator output is high, the value on the thumbwheel switch is activated.

Another way to control the membrane potential is by using the EXT. COMMAND input. Any voltage applied to this input will be proportionately applied to the membrane. For example, if you applied a sine wave command potential you would see a sine wave membrane potential. The scaling factor is set on the front-panel EXT. COMMAND SENSITIVITY switch. It is important to note that if you have no external command source connected you should set the EXT. COMMAND SENSITIVITY to the OFF position. Otherwise, the open-circuited input will pick up spurious transients.

Electrode Capacitance Compensation

Switch OFF all external command sources. Set ß = 100. Dial up 010.0 mV on the thumbwheel switch and press the OSC. button. In the Outputs section, set OUTPUT SELECT = I.

You will notice large positive and negative spikes on the current waveform. These represent the current required to charge the electrode capacitance to each potential. These spikes can be almost fully compensated for by the Fast and Slow Capacitance Compensation controls.

Eliminate the major portion of the spikes by iteratively adjusting the FASTτ and FAST MAG. controls. The spikes should almost disappear into the noise. If slow residual spikes are present, they can normally be eliminated by iteratively adjusting the SLOWτ and SLOW MAG. controls.

Whole-Cell Recording

So far you have been recording from an attached patch. In order to watch the progress from patch recording to whole-cell recording, switch R_{SEAL} TEST to 20 mV OSC. Set the OSCILLATOR FREQ. to 100 Hz, ß = 1, OUTPUT SELECT = I, α = 1 and -3db FREQ. = 10 kHz. Set the HOLDING POTENTIAL to -50 mV so that the cell will hopefully be held near its resting potential when the patch is ruptured.

To record from the whole cell you must rupture the patch (or change the model cell to the CELL position). There are two ways to rupture the patch. You can either apply a brief pulse of suction, or you can use the ZAP control. Zap puts a 1.5 V pulse onto the patch for a duration you select. Start with DURATION set to 0.1 ms. Press the TRIG. button. Successful rupturing of the patch will be accompanied by large capacitance-charging current transients in response to the 20 mV seal-test command. If the patch does not rupture, try increasing the Zap duration. In some preparations Zap may not work; instead of rupturing the patch it will destroy the seal. This will be obvious, because instead of seeing exponentially decaying current transients (to charge and discharge the membrane capacitance) you will see large current steps which have little if any decay.

Once you are in whole-cell recording mode you can switch OFF the R_{SEAL} TEST and adjust the HOLDING POTENTIAL until the current is zero. This corresponds to the resting membrane potential. All commands, either from the Step Command or the External Command, will be relative to this potential.

When applying voltage steps in whole-cell mode there is a large current transient to charge the membrane capacitance. This can be compensated for by adjusting the SERIES RESISTANCE and WHOLE-CELL CAP. controls in the Capacitance Compensation section. Whole-Cell Capacitance Compensation is operative only with the standard CV-4-1/100U headstage. Series Resistance Compensation is operative with all the headstages.

Switch the STEP COMMAND to -20 mV, OSC., 100 Hz. Advance the WHOLE-CELL CAP. control one turn, and then iteratively adjust the SERIES RESISTANCE and the WHOLE-CELL CAP. controls to eliminate the large slow transient. This step is not possible if you are using an 0.1/100U headstage. The setting on the SERIES RESISTANCE control corresponds to the combined electrode and access resistance; the setting on the WHOLE-CELL CAP. control is the membrane capacitance.

Use the FASTτ and FAST MAG. controls to minimize the fast transient. You may need to use the SLOWτ and SLOW MAG. controls as well.

If you are going to be looking at membrane currents of several nanoamps or more, you will need to compensate for the error due to the voltage drop across the electrode resistance.

To do so, after eliminating the transients as described, slowly advance the % COMPENSATION control located in the Correction Potentials section. It is normal for the current noise to increase because compensating for the series resistance actually increases the recording bandwidth.

As you increase the % Compensation there will likely be a tendency towards oscillation. This can usually be removed by carefully readjusting the FAST MAG. control. If you iteratively increase the % COMPENSATION and fine tune the FAST MAG., you will often be able to achieve 80% compensation or more. 80% compensation means that the 50 mV voltage drop that would be caused by a 10 nA current in a 5 MΩ electrode will only cause a 10 mV error in the clamp potential (*i.e.*, only 20% of the uncompensated error).

In some cells, using the LAG control can dramatically increase the maximum achievable % Compensation. It improves stability by cutting out the compensation at high frequencies. Therefore, you should use this control as little as possible if you are looking at very fast currents.

Current Clamp

The normal mode of operation of a patch clamp is voltage clamp. In voltage-clamp mode the membrane potential is controlled and the current needed to maintain that control is recorded.

It is often useful to allow the membrane potential to change freely. This is done in current-clamp mode in which the membrane current is controlled (often at zero) while the membrane potential is recorded.

Current-clamp mode (I-CLAMP) can be used with all values of ß, although it is most often used with ß = 1 or ß = 0.1. You should be aware that voltage recording (current-clamp) in the Axopatch-1D or any other patch clamp is not nearly as fast as voltage recording in a conventional current clamp such as the Axoclamp or Axoprobe.

In I-CLAMP mode, all of the voltage commands become current commands of amplitude 10 ÷ ß pA/mV. Thus with ß = 1, a 10 mV voltage command corresponds to 100 pA.

Typically, you will change to I-CLAMP mode after establishing whole-cell recording in V-CLAMP mode. Switch OFF all commands except the HOLDING POTENTIAL, set the % COMPENSATION to OFF, and reset all of the controls in the Capacitance Compensation section to Zero. Choose V_m on the OUTPUT SELECT switch. Change the MODE selector to the I = 0 position. You are now in current clamp, but all commands are being ignored and the current is being clamped at zero. This is your opportunity to turn the HOLDING POTENTIAL down to zero. If you did so while you were still in V-CLAMP mode you would have clamped the cell at zero instead of at its rest potential. If you waited until after you had switched into I-CLAMP mode you would have had an unwanted current command corresponding to the setting of the Holding Potential.

After turning the HOLDING POTENTIAL down to zero, switch into I-CLAMP mode. Switch the STEP COMMAND to OSC. and the OSCILLATOR FREQ. to 10 Hz. Increase the magnitude on the thumbwheel switch until you see an exponential voltage response. This is the voltage developed as the membrane capacitance charges.

Expand the oscilloscope trace to magnify the initial part of the voltage response. It may help to set the OSCILLATOR FREQ. to 100 Hz and to set the -3 dB FREQ. to 10 kHz. The fast initial step can be made faster by increasing the setting of the FAST MAG. control. The fast initial step is the voltage drop across the electrode. This can be eliminated from the recording by advancing the SERIES RESISTANCE control. This is equivalent to using the Bridge Balance control on conventional current-clamp instruments.

To return to V-CLAMP, first select the I = 0 position. Set the HOLDING POTENTIAL dial setting to the resting membrane potential of your cell and then select V-CLAMP.

Other Controls

Output Calibration

The OUTPUT CAL. adds a 100 mV level to all outputs. Switch from OFF to CONT. and you should see the oscilloscope trace shift by 100 mV. Note that this 100 mV level is not applied to the cell; it is summed into the outputs to assist you in calibrating external recording apparatus. The 100 mV level can be gated on and off by an external logic pulse. Alternatively, you can connect a signal to the EXT. CAL. SIGNAL input and a proportional signal will be summed into the outputs.

Gain and Frequency Senders

The setting of the rotary GAIN and FREQUENCY controls in the Outputs section can be measured on the GAIN and FREQUENCY SETTING connectors on the rear panel. Connect a digital voltmeter to the GAIN SETTING connector. Note how the voltage changes as you rotate the GAIN control. You can modify your computer acquisition program to read and record these values automatically. The pCLAMP program from Axon Instruments are already designed to record these values.

Current Convention

The current convention used on the Axopatch-1D is that current out of the electrode is positive.

Boost Box

The inherent bandwidth of a 50 GΩ resistor is only a few tens of Hertz, while for single-channel recording a bandwidth of many kilohertz may be required. Special highpass filter circuitry is used to boost the bandwidth of the headstage output.

The boost circuitry is located in a special box inserted into the headstage cable. It is not put into the main unit since its design and calibration depend on the headstage configuration and componentry respectively. It is pre-tuned at the factory. However, you may need to re-tune it every 6-12 months. The tuning procedure is described in the *Headstage* section of the **REFERENCE GUIDE** chapter.

Except when it is being re-tuned, the boost box should be plugged into the receptacles on the rear panel. Parking the boost box in this way is a mechanical convenience; it has no bearing on the electrical performance.

Conclusion

This chapter is designed to help you get started. We recommend that after you have worked your way through this chapter, you read the **REFERENCE GUIDE**. There are many details and insights in the **REFERENCE GUIDE** that you must be aware of to make best use of your Axopatch-1D.

REFERENCE GUIDE

The controls and operation of the Axopatch-1D are described in this chapter. The topics are arranged in alphabetical order.

Audio Monitor

The audio monitor is a voltage controlled oscillator (VCO) that drives a small speaker. The control voltage (V_c) is either V_m, I, or an external signal depending on the setting of the OUTPUT SELECT switch. As V_c varies so too does the pitch of the audio tone.

When $V_c = 0$ the frequency is about 2.25 kHz. This frequency drops by approximately 3 octaves as V_c decreases to -100 mV.

The Audio Monitor enables changes in the electrode potential or current to be recognized without having to look at the oscilloscope or panel meters. During seal formation a change in tone can be clearly heard as the test pulse becomes smaller. There is also an abrupt change in tone when a new patch pipette first touches the solution. Thus, the patch pipette can be lowered towards the preparation very rapidly and stopped as soon as the tone change indicates contact with the solution.

An earphone can be plugged into the phone jack. This disables the speaker.

After seal formation, the volume control should be turned fully counterclockwise until it clicks off.

Suggested Use

Set the volume to a quiet level; select I on the OUTPUT SELECT switch. Identify successful seal formation by the change in the audible response to the test pulses. After seal formation, switch the volume control off.

Bath Headstage

In some experiments it is desirable to make voltage measurements relative to a reference point in the bathing solution rather than relative to ground. (These conditions may include precision measurements during changes of temperature or ion content of the saline, or cases of restricted access from the extracellular space to the grounding point.)

All measurements are normally made relative to the system ground. However, if a BH-1 bath headstage is plugged into the rear-panel connector, measurements are automatically made relative to the potential recorded by this headstage.

To minimize added noise, the bandwidth of the BH-1 headstage is limited to 1 kHz. Added noise is further prevented by recording the bath potential at x20 gain using a FET-input low-noise amplifier. To keep the noise low, the patch pipette used for recording bath potential should be no more than a few kΩ.

The bath headstage cannot be used for passing current.

The case of the BH-1 headstage is grounded. The gold-plated case connector and the yellow ground output are both connected to the same signal ground. You should not normally use these outputs.

The red connector is the electrode input.

To minimize line-frequency pickup, the bath and main headstage cables should be loosely twisted or tied together as they run from the Axopatch-1D to the preparation.

The bath potential is provided at x20 gain on the V_{BATH} output.

Non-use

If you are not using the bath headstage for a particular experiment, you should either ground the input of the bath headstage or disconnect the bath headstage from the rear panel of the Axopatch-1D.

Capacitance Compensation

Pipette Capacitance Compensation

The Fast and Slow Compensation controls are used to charge the pipette capacitance (C_p) during a voltage step.

A simplified circuit of the Fast and Slow compensation controls is shown in the figure. This circuit presumes that there is no membrane current, either because the pipette is open circuit, or because the pipette current is clearly distinguishable on the basis of time course from the current required to charge C_p.

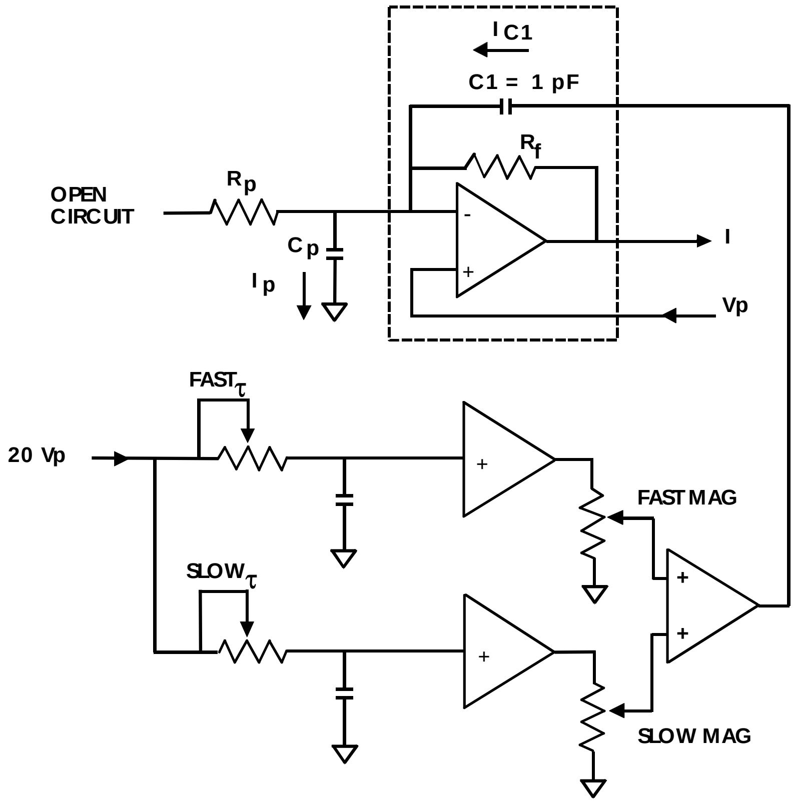
When the command potential (V_p) changes, current I_p flows into C_p to charge it to the new potential. If no compensation is used, I_p is supplied by the feedback resistor (R) resulting in a large transient signal on the output (I).

By properly setting the FAST and SLOWτ and MAG. controls, a current (I_{C1}) can be induced in capacitor C1 (connected to the headstage input) to exactly equal I_p. In this case no current need be supplied by R, and there is no transient on the output.

The Fast controls compensate that part of C_p that can be represented by a lumped capacitance at the headstage input. This is the major part of C_p. A small amount of C_p can only be represented as a capacitor with a series resistance component. This takes longer to charge to its final value. It is compensated by the Slow controls.

Suggested Use

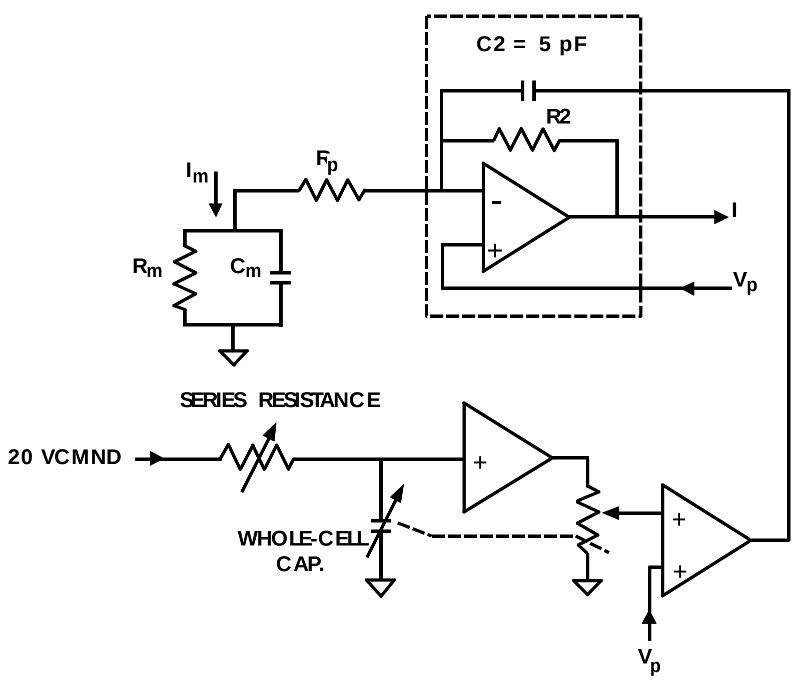
After a seal is made, generate a repetitive command potential. Observe I. Iteratively adjust the FASTτ and FAST MAG. controls to minimize the fast transient. Use the SLOWτ and SLOW MAG. controls to minimize any residual slow component.



Membrane Capacitance Compensation

The SERIES RESISTANCE and WHOLE-CELL CAP. controls are used to charge the membrane capacitance (C_m).

A simplified circuit of these controls is:



Assume that the Fast and Slow compensation controls have already been set to compensate for C_p. By appropriately adjusting the SERIES RESISTANCE and WHOLE-CELL CAP. controls, the current injected through C2 will supply the transient membrane current (I_m). Note that the WHOLE-CELL CAP. Compensation control is not operative with the 0.1/100U headstage.

These adjustments do not in fact alter the time constant for charging the membrane. What they do is offload the burden of this task from the feedback resistor, R2. This can have two benefits:

1. In many cells, even a small command voltage of a few tens of millivolts can require such a large current to charge the membrane that it cannot be supplied by R2. The headstage output saturates for a few hundred microseconds or a few milliseconds, thus extending the total time necessary to charge the membrane.

2. The series-resistance correction circuitry (see the *Series Resistance* section), which can in fact speed up the charging of the membrane, increases the current-passing demands on R2. By moving the pathway for charging the membrane capacitance from R2 to C2, the series-resistance circuitry can operate without causing R2 to saturate.

ß = 0.1 for Large Cells

If R2 were small enough, it would not be necessary to have the ability to charge the membrane capacitance via C2. All of the current required to charge the membrane capacitance could be supplied via R2. This will often be the case when using the ß = 0.1 option (R2 = 50 MΩ, C2 = 0 pF). When using the ß = 0.1 option, the WHOLE-CELL CAP. Compensation control is disabled. In lieu of this, the maximum current that can be passed is ten times larger than when ß = 1. (The maximum current with ß = 0.1 is 200 nA.)

The ß = 0.1 option is required for cells whose membrane capacitance is greater than 100 pF. The reason why the ß = 0.1 option can charge cells with larger capacitances is that the required current can be supplied directly through R2.

You might postulate that an alternative option would be to have ß = 1 (*i.e.*, R2 = 500 MΩ) coupled with a tenfold larger C2 (*i.e.*, C2 = 50 pF). This is not possible because the added capacitance adds grossly to the noise.

Suggested Use

After the seal has been ruptured, generate a repetitive command potential.

To eliminate the capacitance-charging transient:

1. Turn the WHOLE-CELL CAP. control to read several pF.
2. Turn the SERIES RESISTANCE control to eliminate the initial step of the transient.
3. Repeat steps 1 and 2 until the transient is minimized.
4. Re-adjust the Fast and Slow Compensation controls.

This procedure leads to unique settings of the SERIES RESISTANCE and WHOLE-CELL CAP. controls corresponding to the electrode and cell being clamped.

Next, you would usually set the % COMPENSATION controls as described in the *Series Resistance* section.

Limitations

The measurement of series resistance (R_p) and cell capacitance (C_m) is only accurate if the membrane resistance (R_m) is significantly greater than R_p.

Command Potentials

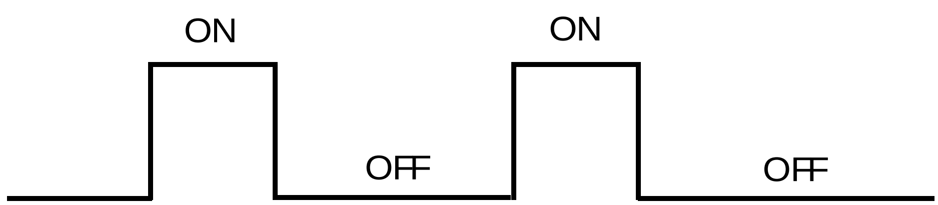
Command potentials can be obtained from several internal sources as well as from some external sources.

Oscillator

The internal oscillator can be used to activate the internal command potentials.

The three available frequencies are 1 Hz, 10 Hz and 100 Hz. The frequencies are accurate to within 10% of nominal values. The internal oscillator is not meant to be your primary timing source for experiments. It is meant to be an easy-to-use aid during the set-up phases.

The oscillator is on for 0.33 of each period and off for 0.67 (see figure below). Thus, the on and off states can be easily distinguished.



Step Command

The STEP COMMAND generator uses a digital-to-analog converter for accuracy and repeatability. The maximum magnitude on the thumbwheel switch is 199.9. Plus (+) corresponds to positive pipette voltage and current. Minus (-) corresponds to negative pipette voltage and current.

The STEP COMMAND can be activated three ways: 1) Press the CONT. pushbutton. This causes continuous activation of the command. 2) Press the GATE pushbutton. This allows the value on the thumbwheel switch to be gated on by a logic high level on the rear-panel STEP GATE input. 3) Press the OSC. pushbutton. The internal oscillator gates the command at the selected frequency.

When the OFF pushbutton is pressed, the command is zero irrespective of the value on the thumbwheel switch.

Holding Potential

This control allows the membrane potential to be shifted during voltage clamp to a value in the range ±200 mV. In current clamp there is a corresponding DC current. Turn the control fully counterclockwise for a zero command. Use the toggle switch to set the polarity or to switch off.

R_{SEAL} Test

The R_{SEAL} TEST command generator is a quick source of test pulses to be used during seal formation. Only 0.2 mV or 20 mV can be selected and these are gated by the internal oscillator.

Ext. Command Sensitivity

This control sets the attenuation of an external command applied to the rear-panel input. There are three positions.

1. 20 mV/V. General-purpose setting. Allows ±200 mV command from ±10 V source.
2. 1 mV/V. Large attenuation useful for seal-formation test pulses. Allows ±10 mV command from a ± 10 V source.
3. OFF. Overrides external command. Always use the OFF position when there is no external source connected to the rear-panel input. Otherwise, this command input will not be internally grounded and it is likely that transients will be picked up by this input and converted into unwanted transient signals.

Speed Test

SPEED TEST injects a current into the headstage input via small capacitors in the headstage. This is used for verifying the dynamic response of the headstage. If necessary, the controls in the headstage boost box can be adjusted to tune the response.

There are three positions.

1. OSC. Current is gated by the internal oscillator. This position is disabled if the oscillator frequency is 1 Hz.
2. EXT. A waveform applied to the Speed Test input is transformed into a proportional injected current.

   The injection-capacitor current is the derivative of the voltage across it. To preserve the waveshape, the command waveform is passed through an integrater before being applied to the capacitor.
3. OFF. Overrides external input.

The magnitude of the injected current depends on ß. When ß = 100 or ß = 0.1, the associated injection capacitor is 0.5 pF. The injected current is approximately 100 pA in the OSC. position, and for external inputs the proportionality factor is approximately 100 pA/V.

When ß = 1, the associated injection capacitor is 5 pF. The injected current is approximately 1 nA in the OSC. position, and for external inputs the proportionality factor is approximately 1 nA/V.

When ß = 0.1, the external input must be used for tuning the dynamic response. See the manual supplied with the ß = 0.1/100U headstage for this procedure.

Command Summing

Commands from all active sources sum linearly.

Current and Voltage Conventions

The terminology used in this discussion applies to all amplifiers manufactured by Axon Instruments.

Positive Current

The flow of positive ions *out* of the headstage into the microelectrode and out of the microelectrode tip into the preparation is termed positive current.

Inward Current

Current that flows across the membrane, from the outside surface to the inside surface, is termed inward current.

Outward Current

Current that flows across the membrane, from the inside surface to the outside surface, is termed outward current.

Positive Potential

The term *positive potential* means a *positive* voltage at the headstage input with respect to ground.

Transmembrane Potential

The *transmembrane potential* (V_m) is the potential at the inside of the cell minus the potential at the outside. This term is applied equally to the whole-cell membrane and to membrane patches.

Depolarizing / Hyperpolarizing

The resting V_m value of most cells is negative. If a positive current flows into the cell, V_m initially becomes less negative. For example, V_m might shift from an initial resting value of -70 mV to a new value of -20 mV. Since the absolute magnitude of V_m is smaller, the current is said to *depolarize* the cell (*i.e.*, it reduces the "polarizing" voltage across the membrane). This convention is adhered to even if the current is so large that the absolute magnitude of V_m becomes larger. For example, a current that causes V_m to shift from -70 mV to +90 mV is still said to depolarize the cell. Stated simply, *depolarization* is a *positive* shift in V_m. Conversely, *hyperpolarization* is a *negative* shift in V_m.

Whole-Cell Voltage and Current Clamp

Depolarizing / Hyperpolarizing Commands

In whole-cell voltage clamping, a *positive* shift in the command voltage causes a positive shift in V_m and is said to be *depolarizing*. A *negative* shift in the command voltage causes a negative shift in V_m and is said to be *hyperpolarizing*.

Transmembrane Potential *vs*. Command Potential

In whole-cell voltage clamp, the command potential controls the voltage at the tip of the intracellular voltage-recording microelectrode. The transmembrane potential is thus equal to the command potential.

Inward / Outward Current

In a cell generating an action potential, depolarization is caused by a flow of positive sodium or calcium ions *into* the cell. That is, *depolarization* in this case is caused by an *inward* current.

During intracellular current clamping, a depolarizing current is a *positive* current out of the microelectrode tip into the interior of the cell. This current then passes through the membrane *out* of the cell into the bathing solution. Thus, in intracellular current clamping, a *depolarizing (positive)* current is an *outward* current.

An *inward* sodium current flows in some cells after a depolarizing voltage step. When the cell is voltage clamped, the sodium current is canceled by an equal and opposite current flowing into the headstage via the microelectrode. Thus it is a *negative* current. When two-electrode voltage clamping was first used in the early 1950's, the investigators chose to call the *negative* current that they measured a *depolarizing* current because it corresponded to the depolarizing sodium current. This choice, while based on sound logic, was unfortunate because it means that from the recording instrument's point of view, a negative current is *hyperpolarizing* in intracellular current-clamp experiments but *depolarizing* in voltage-clamp experiments.

To prevent confusion, Axon Instruments has decided to always use current and voltage conventions based on the instrument's perspective. That is, the current is always unambiguously defined with respect to the direction of flow into or out of the headstage. Some instrument designers have put switches into the instruments to reverse the current and even the command voltage polarities so that the researcher can switch the polarities depending on the type of experiment. This approach has been rejected by Axon Instruments because of the real danger that if the researcher forgets to move the switch to the preferred position, the data recorded on the computer could be wrongly interpreted. Axon Instruments believes that the data should be recorded unambiguously.

Patch Clamp

By design, the patch-clamp command voltage is positive if it increases the potential inside the micropipette. Whether it is hyperpolarizing or depolarizing depends upon whether the patch is "cell attached", "inside out" or "outside out". The patch-clamp pipette current is positive if it flows from the headstage through the tip of the micropipette into the patch membrane.

Cell-Attached Patch

The membrane patch is attached to the cell. The pipette is connected to the outside surface of the membrane. A *positive* command voltage causes the transmembrane potential to become more negative, therefore it is *hyperpolarizing*. For example, if the intracellular potential is -70 mV with respect to 0 mV outside, the potential across the patch is also -70 mV. If the potential inside the pipette is then increased from 0 mV to +20 mV, the transmembrane potential of the patch hyperpolarizes from -70 mV to -90 mV.

From the examples it can be seen that the transmembrane patch potential is inversely proportional to the command potential, and shifted by the resting membrane potential (RMP) of the cell. A positive pipette current flows through the pipette, across the patch membrane into the cell. Therefore a *positive* current is *inward*.

Inside-Out Patch

The membrane patch is detached from the cell. The surface that was originally the inside surface is exposed to the bath solution. Now the potential on the inside surface is 0 mV (bath potential). The pipette is still connected to the outside surface of the membrane. A *positive* command voltage causes the transmembrane potential to become more negative, therefore it is *hyperpolarizing*. For example, to approximate resting membrane conditions of V_m = -70 mV, the potential inside the pipette must be adjusted to +70 mV. If the potential inside the pipette is increased from +70 mV to +90 mV, the transmembrane potential of the patch hyperpolarizes from -70 mV to -90 mV.

From the example it can be seen that the transmembrane patch potential is inversely proportional to the command potential. A positive pipette current flows through the pipette, across the patch membrane from the outside surface to the inside surface. Therefore a *positive* current is *inward*.

Outside-Out Patch

The membrane patch is detached from the cell in such a way that the surface that was originally the outside surface remains exposed to the bath solution. The potential on the outside surface is 0 mV (bath potential). The pipette interior is connected to what was originally the inside surface of the membrane. A *positive* command voltage causes the transmembrane potential to become less negative, therefore it is *depolarizing*. For example, to approximate resting membrane conditions, assuming that V_m = -70 mV, the potential inside the pipette must be adjusted to -70 mV. If the potential inside the pipette is then increased from -70 mV to -50 mV, the transmembrane potential of the patch depolarizes from -70 mV to -50 mV.

The membrane potential is directly proportional to the command potential. A positive pipette current flows through the pipette, across the patch membrane from the inside surface to the outside surface. Therefore a *positive* current is *outward*.

Summary

1. *Positive* current corresponds to:

Cell-attached patch	patch inward current
Inside-out patch	patch inward current
Outside-out patch	patch outward current
Whole-cell voltage clamp	outward membrane current
Whole-cell current clamp	outward membrane current

2. A *positive* shift in the command potential is:

Cell-attached patch	hyperpolarizing
Inside-out patch	hyperpolarizing
Outside-out patch	depolarizing
Whole-cell voltage clamp	depolarizing

3. The correspondence between the command potential (V_{CMND}) and the transmembrane potential (V_m) is:

Cell-attached patch	Vm = RMP - Vc
Inside-out patch	Vm = - Vc
Outside-out patch	Vm = Vc
Whole-cell voltage clamp	Vm = Vc

Current Clamp

In current-clamp mode, the Axopatch-1D can be used similarly to a conventional microelectrode amplifier. The error due to electrode voltage drop during current-passing can be compensated by using the SERIES RESISTANCE control (analogous to "Bridge Balance" in a conventional microelectrode amplifier). The response speed can be improved by using the Fast and Slow Capacitance Compensation controls.

During current clamp, all of the voltage commands become current commands. The proportionality factor is (10 ÷ ß) pA/mV. Thus, if ß = 1, a 10 mV command would correspond to a 100 pA current command. For an external command sensitivity of 20 mV/V a maximal 10 V input would produce a maximal 200 mV command. Thus, the maximum external current command would be 200 mV x 10 ÷ pA/mV = 2000 pA.

Membrane Potential

In voltage-clamp mode, V_m is simply equal to the command potential. In current-clamp mode, V_m is quite difficult to derive. It is normally equal to the pipette potential but several corrections are made. These are 1) removal of the pipette offset set on the JUNCTION NULL controls 2) removal of the bath potential, if measured, and 3) subtraction of the error due to the current-induced voltage drop across the pipette.

To avoid introducing errors, you should not change the JUNCTION NULL controls after you switch from V-CLAMP to I-CLAMP. That is, if you were using HOLD, leave HOLD on. Do not reset the MANUAL JUNCTION NULL while in I-CLAMP whether HOLD is on or not.

Whole-Cell Current Clamp

Current-clamp can be used from the very beginning of the experiment, or you can switch into current-clamp mode at any time. However, if whole-cell recording has been established, special care must be taken when switching between current-clamp and voltage-clamp modes. This is because in voltage-clamp the holding potential must be set to a negative value to hold the cell at rest, whereas in current-clamp, the holding potential (which acts as a DC current command) must be equal to zero for the cell to be at rest. The intermediate "I = 0" mode is provided to allow you to conveniently switch between current and voltage clamp.

To switch from voltage clamp to current clamp:

1. Select I = 0 mode.
2. Turn off the HOLDING POTENTIAL and any other commands.
3. Select I-CLAMP mode.

To switch from current-clamp to voltage clamp:

1. Select I = 0 mode.
2. Turn the HOLDING POTENTIAL control to the resting membrane potential. Switch off all other commands.
3. Select V-CLAMP mode.

Series Resistance

When current flows across the series resistance (of the electrode plus access resistance), there is a voltage drop which appears as if it were part of the membrane potential. This error voltage is only a problem in whole-cell current clamping where the current is several nA and the patch pipette resistance is several MΩ.

The error voltage can be subtracted from the recorded membrane potential by using the SERIES RESISTANCE control in the Capacitance Compensation section. To set this control, you must pulse the cell with a repetitive step current. Ideally, the error voltage will be visible on the V_m output (SCALED OUTPUT set to V_m) as a fast component at the beginning of the step followed by a slower charging of the membrane potential. In this case, advance the SERIES RESISTANCE control until the fast component is eliminated from the V_m output. The value of the series resistance can be directly read from the dial. The sensitivity is 10 MΩ/turn.

In practice, the difference is speed between the two components of the V_m response is often not sufficiently great to allow this technique to be used. If this is the case, the best you can do is to estimate the series resistance and set the value accordingly. Of course, the best technique by far is simply to use a sufficiently low patch pipette resistance so that the product of the resistance and the commanded current is at most a few mV.

In Current-Clamp mode, the Series Resistance compensation is based on the **commanded** current, not the actual current. This has the advantage of not adding noise to the V_m recording. The current measurement, however, is a measurement of the **actual** patch pipette current.

Capacitance Compensation

The speed of the pipette depends on the pipette resistance (R_p) and the input capacitance (C_p). (See the section on *Pipettes* for information on how to patch pipettes for low resistance and capacitance.) As a first-order approximation, the electrode time constant depends on the product R_p C_p. The Fast and Slow Capacitance Compensation controls can be used to electronically reduce the effective value of C_p, and thus the pipette time constant. This is achieved by injecting a transient current into the headstage input to charge and discharge C_p during signal changes.

If the pipette capacitance compensation is incorrectly set, it will be difficult to distinguish the component of the step response that is due to charging the pipette capacitance from the component that is due to charging the membrane capacitance. Thus, the value of the membrane capacitance derived from the time constant of the step response may be an overestimate.

The most convenient way to set the pipette capacitance compensation in voltage-clamp mode, as described in the *Capacitance Compensation, Suggested Use* section of the **REFERENCE GUIDE** chapter. Once the controls are optimally set, switch back to current clamp.

Limitations

Current-clamp in the Axopatch-1D or any other patch-clamp amplifier is not as fast or stable as current-clamp amplifier (*i.e.,* voltage recording) in a conventional current clamp such as the Axoclamp or Axoprobe. This is because of the significant differences in the design of the headstages.

In a conventional current-clamp amplifier, the headstage is designed as a voltage follower. Current is injected through a resistor and the pipette voltage is continuously recorded.

In a patch-clamp amplifier, the headstage is designed as a current follower. The pipette voltage is controlled while the pipette current is measured. To simulate current-clamp, a feedback circuit in the main unit automatically adjusts the pipette voltage to keep the pipette current at the desired value. Like any feedback circuit, the stability is compromised if the open-loop gain is too high. When the headstage is grounded by a pipette, the voltage gain of the headstage is very nearly equal to the value of the feedback resistor divided by the value of the pipette resistance. For ß = 100 (R_f = 50 GΩ) and a pipette resistance of 1 MΩ, this voltage gain is 50,000. In order to guarantee stability with pipette resistances as low as 1 MΩ, the current-clamp circuitry must be deliberately slowed, which compromises the response time for high-resistance pipettes. For low-resistance pipettes the main problem is stability. In the extreme case of a zero-resistance pipette (*i.e.*, a directly grounded input), the enormous voltage gain of the headstage guarantees instability. To compromise between the two conflicting requirements of speed and stability, the Axopatch-1D has been designed for stability at all values of ß with pipette resistances down to 1 MΩ. Pipette resistances much smaller than 1 MΩ will probably cause oscillation during current clamp.

Current Range in Current Clamp Mode

The current injected into the pipette is (10 ÷ ß) pA/mV. The command sensitivity and the maximum current that can be injected through electrodes of 100 MΩ or less are given in the table.

	Command Sensitivity	Maximum Current	Max. Current Ext. Command	Series Resistance Compensation Range
ß = 0.1	100 pA/mV	60 nA	20 nA	10 MΩ
ß = 1	10 pA/mV	6 nA	2 nA	100 MΩ
ß = 100	0.1 pA/mV	0.6 nA	.2 nA	-

Since the maximum voltage command produced by the Holding Potential circuit is ±200 mV, the maximum current that can be injected using the Holding Potential is 2 nA with ß = 1, and 20 nA with ß = 0.1. Additional current, up to the maximum indicated above, can be injected using an external voltage source. For example, simultaneous application of maximum step command, holding potential and external command will result in 6 nA of current at ß =1.

Suggested Use

Observe V_m on the oscilloscope. Set all the CAPACITANCE COMPENSATION controls to zero. Under whole-cell recording conditions switch from V-CLAMP mode to I = 0 mode. If you have not already done so, select ß = 1. Do not alter any of the JUNCTION NULL controls. Switch off all commands including the HOLDING POTENTIAL. Now switch into I-CLAMP mode.

Switch the STEP COMMAND to OSC., 10 Hz. Increase the magnitude on the thumbwheel switch until you see an exponential voltage response (due to charging of the membrane capacitance).

There will be a small fast voltage step at the start and finish of the current step. This is the voltage developed across the pipette during current flow. Advance the SERIES RESISTANCE control (in the Capacitance Compensation section) until these fast voltage steps are just eliminated. The residual transients at the start and finish of the current steps are due to the finite response speed of the pipette. These transients can be minimized by correctly setting the CAPACITANCE COMPENSATION controls. The setting of the SERIES RESISTANCE control corresponds to the sum of the pipette and access resistances.

Advance the FAST MAG. control in the *Capacitance Compensation* section to minimize the residual transients.

Alternatively, reset the SERIES RESISTANCE control to zero. Increase the oscillator frequency to 100 Hz. Now the voltage drop across the pipette should be clearly visible. Advance the FAST MAG. control as far as possible without introducing an overshoot in the step response. In some cases, the other three capacitance compensation controls can be used to fine tune the response. This setting is optimal for current passing and is also optimal for recording potentials at the tip of the pipette.

Grounding and Hum

A perennial bane of electrophysiology is line-frequency pickup (noise), often referred to as hum. Hum can occur not only at the mains frequency but also at multiples of it.

The Axopatch-1D has inherently low hum levels (less than 20 µV peak-to-peak). To take advantage of these low levels great care must be taken when integrating the Axopatch-1D into a complete recording system. The following procedures should be followed.

1. **Ground the preparation bath by directly connecting it to the gold ground connector on the back of the headstage. There should be no other ground connections to the preparation bath.**

2. Place the Axopatch-1D in a position in the rack where transformers in adjacent equipment are unlikely to radiate into its electronics. The most sensitive part of the electronics is the right-hand side looking from the front. A thick sheet of steel placed between the Axopatch-1D and the radiating equipment can effectively reduce induced hum.

3. Initially make only one connection to the Axopatch-1D. This should be to the oscilloscope from the 10 V_m output. After verifying that the hum levels are low, start increasing the complexity of the connections one lead at a time. Leads should not be draped near transformers located inside other equipment. In desperate circumstances, the continuity of the shield on an offending coaxial cable can be broken.

4. Try grounding auxiliary equipment from a ground distribution bus. This bus should be connected to the Axopatch-1D via the yellow banana (4 mm) socket on the rear panel. This socket is connected to the Axopatch-1D's signal ground (*i.e.*, the outer conductors of all the BNC connectors). The signal ground in the Axopatch-1D is isolated from the chassis and power ground.

5. If a bath headstage is used, both headstage cables should run from the Axopatch-1D to the preparation in a bundle. The bundle can be formed either by gently twisting the cables together or by loosely tying them together.

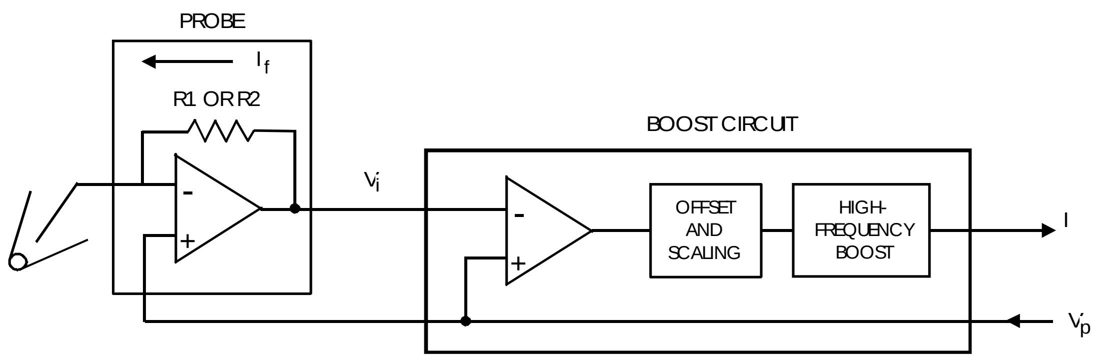
6. Experiment. While hum can be explained in theory (*e.g.*, direct pickup, earth loops), in practice the ultimate theory is the end result. Following the rules above is the best start. One technique that should **not** be used to reduce hum is the delicate placement of cables so that a number of competing hum sources cancel out. Such a procedure is too prone to accidental alteration.

Headstages

Principles of Operation

Patch-clamp headstages are current-to-voltage (CV) converters. That is, the voltage output corresponds to the current input. In contrast, traditional microelectrode amplifier headstages are voltage followers, for which the voltage output corresponds to the voltage input.

The essential parts of the headstage are shown in this figure:



The electrode current is the same as the current through the feedback resistor (R1 or R2). Since the op amp in the probe acts to keep the voltage at its two inputs equal to each other, we know that the potential at its negative input equals V_p. Thus the voltage across R1 is V_i - V_p, which is calculated by the differential amplifier in the boost box. Subsequent amplifiers are used to scale the gain and remove voltage offsets.

A fundamental problem of this circuit when used for patch clamping is that the output bandwidth of the probe is inherently low. To a first approximation, the bandwidth is set by the product of R1 and the stray capacitance across it. For example, if R1 is 50 GΩ and the stray capacitance is 0.1 pF, the bandwidth is about 30 Hz.

To overcome this limitation, the probe output is passed through a high-frequency boost circuit. This circuit has a gain which is proportional to frequency. Because R1 (or R2) does not normally have a first-order response, it is necessary to provide several stages of boost which must be individually matched to R1. The adjustable parameters of the boost circuit are called poles and zeros.

If the Probe Output is Slow, How can Voltage Clamping be Fast?

After a casual consideration, you might conclude that if the output of the probe is slow, the voltage clamping of the pipette must also be slow. The high-frequency boost occurs after-the-fact and cannot influence the clamp speed.

In fact, despite the slow probe output, the voltage clamping of the pipette is very fast. The op amp acts to keep its negative input equal to its positive input and it does this by passing a current (I_f)

through R1 and its associated stray capacitance (CS1). I_f is equal to the pipette current (neglecting strays and bias currents).

As CS1 is made larger, a larger fraction of I_f flows through CS1 but the total value of I_f is unaffected. For larger CS1 values, the op amp output makes smaller voltage excursions to achieve the same I_f value. Thus, even though the op amp output is diminished (in a frequency-dependent way), the clamp quality is substantially unaffected by large CS1 values.

The function of the boost box is to recover the output signal bandwidth that has been lost because of the presence of CS1, thereby allowing a more realistic measurement of the wideband pipette current to be made.

What is Clamped?

Voltage clamping is the intrinsic mode of operation of this type of headstage. The series combination of the electrode and the patch/cell membrane is voltage clamped.

It is assumed that the membrane potential equals the command potential (V_f). This is only true if the current causes a negligible voltage drop across the pipette.

Gain Selection

The feedback resistor can be remotely switched between R1 and R2. There are separate offset, scaling and boost circuits for the two resistors.

The two gains (ß) available in your particular headstage are marked on the probe cover. Use the HEADSTAGE GAIN switch on the front panel of the Axopatch-1D to select one of these values. All CV-4 headstages have a higher gain of 100. The lower gain is either 1 or 0.1.

Which Headstage Should You Use?

Three kinds of CV-4 headstages are available.

CV-4-1/100U This is the standard headstage and the one that is most commonly used. The ß = 100 position is used for single-channel recording. The ß = 1 position is used for whole-cell clamping in small and medium cells. In the ß = position, up to 100 pF of membrane capacitance can be compensated. Assuming that the membrane capacitance is 1 µF/cm^2, this corresponds to a maximum cell diameter of about 60 µm. Another upper limit is that in the ß = 1 position the maximum current that can be passed is about 20 nA. This is more than sufficient for most whole-cell clamping experiments.

CV-4-0.1/100U This headstage is suitable for whole-cell clamping of much larger cells. The maximum current that can be passed in the ß = 0.1 position is about 200 nA. There is no limit to the membrane capacitance because this headstage does not support membrane capacitance compensation (see the *Series Resistance* section).

In the ß = 100 position, this headstage behaves identically to the standard CV-4-1/100U headstage.

CV-4B-0.1/100U This headstage is specialized in two ways for bilayer clamping.

First, the headstage gain can be remotely switched by a logic-level pulse between ß = 100 and ß = 0.1. This makes it possible to force a step change in the membrane potential using the ß = 0.1 position to rapidly charge the large bilayer membrane capacitance, and then switch to the ß = 100 position within a few milliseconds to record the single-channel currents.

Second, the bandwidth in the ß = 100 position has been reduced to about 6 kHz in order to guarantee stability with the huge electrode capacitance presented by the bilayer. Stability is assured for up to several hundred pF. In the other headstages, electrode capacitances in excess of about 20 pF cause a deterioration of the transient response. This is not a problem with patch electrodes because the capacitance is rarely more than two or three pF.

Use of this headstage to clamp bilayers is described in the CV-4B headstage manual.

Tuning CV-4 Headstages

The boost parameters are set at the factory and should only rarely need resetting.

To check or change the settings, it is necessary to inject a rectangular current waveform into the headstage input.

1. Wait 30 minutes for the equipment to warm up.
2. Leave the headstage open circuited.
3. Select SPEED TEST = OSC.
4. At 10 Hz trim adjustments 1-4 in the boost box for the most square response. There is a small amount of intrinsic droop in the injected current similar in effect to AC coupling. This droop should be ignored.
5. At 100 Hz trim adjustments 5-7 for the most square response.

Offset Adjustment for CV-4

Equipment needed:

1. Oscilloscope
2. PATCH-1 model cell
3. CV-4-0.1/100 or CV-4-1/100 headstage
4. Precision screwdriver, 2 mm head

Prior to adjustment, set "% COMPENSATION", "SERIES RESISTANCE", and "WHOLE CELL CAP" fully counter clockwise.

No load condition:

1. Set ß = 100.
2. The PATCH-1 model cell is disconnected from headstage.
3. Wrap headstage in aluminum foil and ground it.
4. Set to V-CLAMP mode.
5. Set Panel Meter to "I".
6. In the OUTPUTS section, set Gain α = 100; -3dB Freq. = 1 kHz; Output Select = I.
7. Connect Scaled Output to oscilloscope, which is set for Line trigger, 2 Volts/Div., 2 ms/Div.
8. Adjust the VC OFFSET potentiometer of Boost Box until the signal is zero on the oscilloscope.
9. Repeat steps 2-8 for headstage gain ß = 1 or 0.1.

With load to headstage:

1. Set headstage gain ß = 100.
2. Connect PATCH-1 model cell in PATCH mode to headstage.
3. Set to V-CLAMP Mode and press "I" switch of panel meter.
4. Adjust Junction Null with the MANUAL knob for zero current as observed on the oscilloscope.
5. Switch to I=0 Mode (current clamp mode).
6. Press "Vm" of panel meter.
7. With the screwdriver, adjust the CC OFFSET potentiometer (C-Clamp) until the meter reads zero millivolts.
8. Repeat the above steps for the headstage gain ß = 1 or 0.1.

Headstage Gain Adjustment

Connect the model cell BATH position to the headstage input and connect the model cell ground to the headstage ground. Select the headstage gain (start with ß = 1). Adjust the panel meter current reading to nearly zero (difficult for ß = 100). Enter the appropriate command voltage with the STEP COMMAND thumbwheel switch (depends upon ß; see below). Press the CONT. button to apply the command voltage continuously across the 10 MΩ resistor in the model cell BATH position. Use the thumbwheel switch to repeatedly change the polarity from positive to negative. Adjust the appropriate gain potentiometer on the boost box to yield the correct difference. For example: if ß = 100 and the panel meter reads +5.00 pA, the +1 mV command should yield +105.0 pA and the -1 mV command should yield -95.0 pA. (Hint: Don't be fanatical about this.) Command voltages:

ß = 1	100 mV	+ and -	10.00 nA
ß = 100	1mV	+ and -	100.0 pA

Case Ground Connector

The metal headstage case and the gold-plated 2 mm socket at the rear of the headstage are connected to ground. Use this ground for grounding the preparation.

No provision is made for driving a shield since using a driven shield around the pipette increases the high-requency noise.

Mounting the Headstage

There are two mounting options provided with the CV-4 headstages.

One is to grip the insulated mounting rod in a manipulator. This is the simplest method and it is often satisfactory.

For maximum mechanical rigidity, you can mount the headstage directly to some manipulators using the acrylic mounting plate located on the bottom of the headstage. Put mounting screws through the four outside holes.

If you are not using the mounting rod, you can remove it by twisting it counterclockwise. If you are not using the mounting plate you can remove it by removing the four screws holding it onto the headstage case.

Cleaning

To clean salt spills from the headstage connectors, wipe with a damp cloth. Avoid spilling liquids on the headstage.

The Teflon input connector should be kept very clean. This can be done effectively by spraying with alcohol or freon.

Static Precautions

The headstage can normally be safely handled. However, if you are in a laboratory where static is high (*i.e.*, you hear and feel crackles when you touch things), you should touch a grounded metal object immediately before touching the headstage.

You should **not** switch off power to the Axopatch-1D when handling the headstage input since this will upset the thermal equilibrium.

Optical Pick-up

The Teflon input connector and the glass walls of the hybrid package inside the headstage are translucent. High intensity light can get through in sufficient strength to activate the input transistors inside the hybrid. Therefore, you should avoid allowing bright light to fall on the input connector. If you notice line-frequency hum on the current record, it could be due to fluctuating light levels from a bright fluorescent light or equivalent. In general, low light levels are not a problem.

Where to Place the CV-4 Boost Box

The CV-4 boost box should be plugged into the violet jacks on the rear panel of the Axopatch-1D. It is not electrically necessary to do this but it is advisable for mechanical and convenience reasons.

Other Precautions

You should switch off the power to the Axopatch-1D before plugging in or removing a headstage.

Pipette Holder

Features

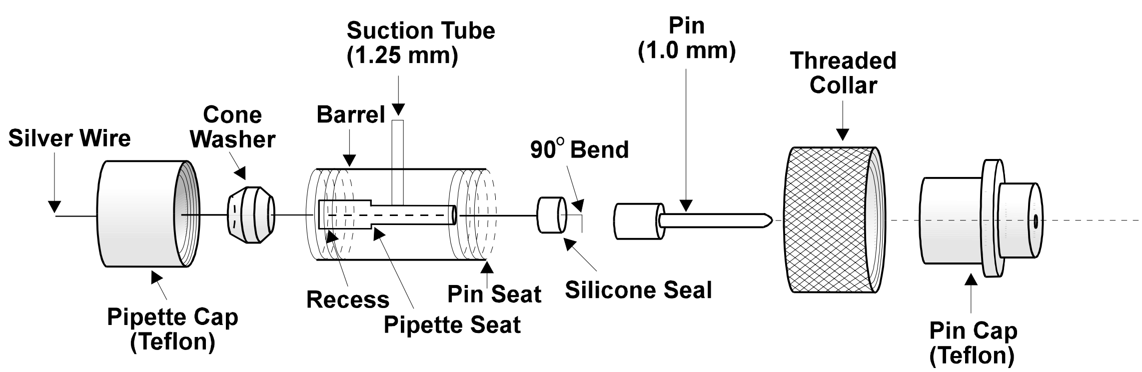
The HL-U series holder provides for enhanced low-noise mechanically stable microelectrode recordings with or without suction. Because the new holder provides a universal fit for a very wide range of pipette diameters and will fit any of our redesigned headstages, it is named the HL-U.

The barrel of the holder is made out of polycarbonate for lowest noise. There are two different barrel lengths. The shorter barrel length contributes less to the operating noise and, therefore, is ideally suited for single channel patch clamp recordings. Although the longer barrel will contribute more to the operating noise, the increased length may provide the needed clearance between the headstage and other components in the experimental setup. Maintenance is simple because the holder can be fully disassembled for cleaning and parts replacement.

Mechanical stability of the pipette is assured in several ways. For example, as the pipette cap is closed, the cone washer is compressed on the pipette from the force applied to the front and back of the cone washer. The holder mates with the special threaded Teflon connector on U-type Axon Instruments headstages and is secured in place with a threaded collar.

The holder is designed to emerge along the long axis of the headstage. A right-angle adapter can be purchased if it is necessary for the holder to emerge at 90° from the headstage.

The HL-U holder is designed to be used with Axon Instruments amplifiers, and fit all U-type CV and HS series of headstages. These headstages have a *threaded* white Teflon collet. To minimize the added noise contributed by the holder in single-channel recording, the holder uses a small (1 mm) pin for the electrical connection and a large amount of insulating Teflon. This noise problem is peculiar to single-channel recording.



Parts

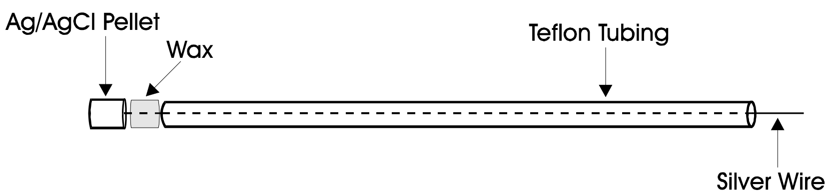
The bore size of the HL-U accepts pipettes with an outer diameter (OD) of 1.0-1.7 mm. Pipettes are secured by a cone washer with an inner diameter (ID) that accommodates the pipette OD. Color-coding aids identification of the four sizes of cone washers: 1.0 mm (orange), 1.3 mm (clear), 1.5 mm (orange) and 1.7 mm (clear). Each HL-U is supplied with two barrel lengths, 16 mm and 28 mm.

It has been shown that a Ag/AgCl pellet offers no greater stability than properly chlorided silver wire. Moreover, the diameter of the Ag/AgCl (1 mm) restricts its use to pipettes with a large ID *i.e.*, > 1.1 mm. Therefore, the HL-U is supplied with 0.25 mm silver wire.

Spare components included with each holder are as follows: one 50 mm length of silver wire, 40 cone washers (10 of each size) and one 70 mm length of silicone tubing. Cut into 2 mm lengths, the silicone tubing will yield approximately 30 replacement silicone seals. Additional cone washers, silicone tubing, pins and silver wire can be purchased from Axon Instruments, as well as optional Ag/AgCl pellet assemblies.

Optional Ag/AgCl Pellets

The HL-U holder will accommodate a 1 mm diameter Ag/AgCl pellet that should provide many months of DC-stable recordings. The inner diameter (ID) of the pipette must be > 1 mm. The silver wire is surrounded by a wax-sealed Teflon tube. This ensures that the electrode solution only contacts the Ag/AgCl pellet. Three pellet assemblies are sold as HLA-003.



Use

Insertion Of Pipette

Make sure the electrode cap is loosened so that pressure on the cone washer is relieved, but do not remove the pipette cap. Push the back end of the pipette through the pipette cap and cone washer until it presses against the pipette seat. Gently tighten the pipette cap so that the pipette is gripped firmly.

To minimize cutting of the cone washer by the sharp back end of the pipette, you can smooth the pipette edges by rotating the back end of the pipette in a bunsen burner flame.

Cleaning

For lowest noise, keep the holder clean. Frequently rinse the holder with distilled water. If more thorough cleaning is required, briefly wash in ethanol or mild soapy water. Never use methanol or strong solvents.

Filling Pipette

Only the taper and a few millimeters of the shaft of the pipette should be filled with solution. The chlorided tip of the wire should be inserted into this solution. Avoid wetting the holder since this will increase the noise.

Silver Chloriding

It is up to you to chloride the end of this wire as required. Chloriding procedures are contained in many electrophysiology texts[1]. Typically the chlorided wire will need to be replaced or rechlorided every few weeks. A simple, yet effective, chloriding procedure is to clean the silver wire down to the bare metal using fine sand paper and immerse the cleaned wire in CHLOROX bleach for about 20 minutes, until the wire is uniformly blackened. This provides a sufficient coat of AgCl to work reliably for several weeks as an internal reference pipette. Drifting or otherwise unstable offsets during experiments is suggestive of the need for rechloriding. The chlorided region should be long enough so that the pipetted solution does not come in contact with the bare silver wire.

Heat smoothing the back end of the pipette extends the life of the chloride coating by minimizing the amount of scratch damage. Another way to protect the AgCl coating is to slip a perforated Teflon tube over the chlorided region.

The chlorided region should be long enough so that the pipette solution does not come in contact with the bare silver wire.

Replacing the Silver Wire

To replace the silver wire, insert the nonchlorided end through the hole of the silicone seal and bend the last 1 mm of wire over to an angle of 90°. Press the wire into the back of the barrel making sure that the silicone seal is flush with the back of the barrel. Slip the threaded collar over the back of the barrel. With the large end of the pin directed toward the bent-over wire screw the pin cap down firmly, but without excessive force. This assures good electrical contact.

Glass Dimensions

Use the HL-U for pipettes with outside diameter (OD) of 1.0-1.7 mm. The optimal dimensions should match the inner diameter (ID) of the four sizes of cone washers, 1.1, 1.3, 1.5 and 1.7 mm. When the pipette OD falls between two sizes of cone washers, the larger size cone washer should be used. For instance, if the pipette OD is 1.6 mm, then use a cone washer with an ID of 1.7 mm.

Adapters

HLR-U right-angle adapters allow the HL-U series holder to emerge at 90° from the headstage. Use the HLR-U with the HL-U holder.

HLB-U BNC-to-Axon adapter allows conventional BNC-type holders to be used with Axon Instruments U-type headstages. Use the HLB-U with all U-type CV and HS headstages (*e.g.,* CV-4-1/100U and HS-2A-x1MGU). These headstages have a threaded white Teflon collet.

Junction Null

The JUNCTION NULL controls are used to compensate for the total offset of the liquid-liquid and liquid-metal junctions in the pipette and bath, and the offset of the probe input amplifier.

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[1]For easy-to-use recipes see Microelectrode Methods for Intracellular Recording and Ionophoresis, by R.D. Purves, London: Academic Press, 1981, p. 51. The Axon Guide. Foster City, CA: Axon Instruments, Inc., 1993, p. 83.

The MANUAL JUNCTION NULL is a ten-turn potentiometer used to add ±250 mV to the pipette command potential (V_p). This is used at the beginning of each experiment to zero the pipette current (I) when the electrode is first touched to the solution.

During and after seal formation, the pipette offset tends to change and the MANUAL JUNCTION NULL may be used again to rezero I. However, it is often more convenient and useful to use the AUTO JUNCTION NULL features.

Auto Junction Null has two operating modes.

1. In TRACK mode, V_p is continuously adjusted to keep I = 0 (or near zero) even though the pipette offset may be changing at a fairly rapid rate. TRACK mode is most often used during seal formation, to stop the I trace from jumping into saturation.

During TRACK mode, I is severely distorted. The effect is very like AC coupling. The Axopatch-1D should **never** be left in TRACK mode once data is being recorded. To remind you that TRACK is on, a red LED glows continuously. The same LED flashes slowly in HOLD mode.

The rate at which Track returns I to zero depends on the pipette resistance. If you are applying a test pulse, you will find that for pipettes of 10 MΩ or less you will see an obvious droop at oscillator frequencies of 10 Hz and you will still probably see some droop at 100 Hz.

2. After making a seal, you can switch from TRACK mode to HOLD mode. In HOLD mode, the value of V_p generated by the Track circuit at the moment you change modes is kept indefinitely. This is extremely useful if you are in a situation in which the pipette offset tends to change permanently during formation of a seal. This does occur in some preparations but the mechanism is not well understood.

Alternatively, after making a seal you can switch from TRACK mode to OFF. When switching from TRACK to OFF one of two things may happen: a) if no permanent offset developed during seal formation, I will be zero; b) if an offset developed during seal formation, there will be a finite I that you can eliminate by resetting the MANUAL JUNCTION NULL.

While in HOLD mode you can tell if a permanent offset developed during seal formation by looking at V_{TRACK} on the main meter. This shows you the extra voltage applied by the Track circuit to keep I = 0. If you are intending for some reason to switch to OFF and you want to avoid I jumping to some nonzero value, you can watch V_{TRACK}, press the RESET button and simultaneously turn the MANUAL control until V_{TRACK} is zero. When you then switch to OFF there will be no visible effect.

Note that in HOLD mode the MANUAL control still affects V_p, so do not touch this control unless you deliberately want to change the set-up.

The Reset button is operative in TRACK and HOLD modes. It has the same effect as a fast track circuit. That is, its mode of operation is the same as TRACK, but the rate at which I is returned to zero is faster.

During RESET and occasionally during TRACK you might see some brief spikes on the current trace. These arise from unavoidable switching transitions in the 16-bit D/A converter used in the Track circuit.

TRACK should only be used after the MANUAL JUNCTION NULL has been initially set (when the pipette first touches the solution). The Track circuit can cope with shifts of about 100 mV away from the setting of the MANUAL JUNCTION NULL. If you do not set the manual control at the start of the experiment, the pipette potential may be outside the tracking range. In this case, you may see the pipette current slowly cycle back and forth, without ever reaching zero. If so, switch off the Track/Hold circuit, set the MANUAL JUNCTION NULL, then return to TRACK.

After a seal is formed the Track circuit becomes very slow in its efforts to keep I = 0. Sometimes you will find that you are ready to switch from TRACK to HOLD but the current has not yet returned to zero. Pressing RESET will hasten this process so that you can be sure that I = 0 before switching into HOLD.

TRACK and HOLD are disabled during current clamp.

Implementation

Digital circuitry is used for the TRACK and HOLD functions. In previous analog designs the output in HOLD mode was held by an analog sample-and-hold that drifted slowly with time. In the digital implementation there is absolutely no drift during HOLD mode.

Errors due to Nonzero Headstage Error Current

It can be shown that for ultrahigh-resistance patches, say 50 GΩ or more, the best strategy after using TRACK is simply to switch to the OFF position. That is, do not use HOLD and do not readjust the MANUAL JUNCTION NULL. The reason is as follows.

The main reason for using the HOLD function is that it automatically compensates for the unexplained permanent offset that develops during seal formation. This is typically less than 10 mV but can be as high as 50 mV.

Without meaning to, the Track/Hold circuitry also tries to compensate for another error. This is the error voltage due to the bias current that is present on the headstage output. This bias current is typically adjusted to zero every few months by turning the appropriate potentiometer inside the boost box until the current reading on the meter is zero. However, small aging and thermal drifts of a few tenths of a pA that develop between adjustments would normally be ignored.

In addition to compensating for the permanent seal offset, the Track circuit also compensates for the error voltage. To do so, the Track circuit applies a correction potential to the pipette to zero the current. A potential is thus developed across the patch. This potential is of sufficient magnitude to force a current equal to the error current to flow through the patch. For a typical error current of 0.1 pA, the required potential across a 50 GΩ patch would be 5 mV. For a 500 GΩ patch the required potential would be 50 mV. If the permanent seal offset is typically around 5 mV, it is clear that the benefit of compensating for it by using the HOLD facility or manually rezeroing is outweighed by the correction potential introduced to correct a 0.1 pA error current in a 50 GΩ patch.

Suggested Use

Lower the pipette until it is in contact with the solution. Use the MANUAL JUNCTION NULL to get I = 0 on the main meter. Select R_{SEAL} = 0.2 mV or 20 mV for ß = 1 or ß = 100, respectively. Set the oscillator frequency to 100 Hz. Select TRACK. Optionally, switch ON the AUDIO MONITOR, OUTPUT SELECT = I. Approach the membrane and form a seal. The track circuitry should keep the current trace on screen all of the time.

The seal resistance is inversely proportional to the size of the current pulses (see the *Seal Formation* section). Measure the size of the current pulses from the initial edge, not from the value after the response has decayed. Notice that as the current response becomes smaller it also shows less droop.

After the seal has formed and the meter shows I = 0 (or near zero), switch to HOLD. You may need to press RESET to hasten the settling of I to near zero.

Proceed with your experiment.

Main Meter

The main panel meter displays one of four signals. Selection is made by pushbutton switches. The four signals are:

1. V_{TRACK}: The output of the Auto Junction Null circuit.
2. V_m: The membrane potential.
3. V_{BATH}: The bath potential. The reading is only nonzero if a bath headstage is connected.
4. I: The electrode current. The reading is in pA for ß = 100 and nA for ß = 1 or 0.1. It is automatically scaled to suit the headstage gain.

Mode

The primary operating mode of the Axopatch-1D is voltage-clamp mode. That is, the pipette potential (V_p) is controlled while the pipette current (I) is measured. An alternative operating mode of the Axopatch-1D is current-clamp. In current clamp mode, I is controlled while V_p is measured.

The discussions in this manual usually assume operation in voltage clamp. The significant changes occurring when current clamp is selected are discussed in the *Current Clamp* section.

The MODE switch has 3 positions:

1. V-CLAMP: Voltage-clamp mode.
2. I = 0: Current-clamp mode, all commands disabled. Current is clamped to zero.
3. I-CLAMP: Current-clamp mode. I is proportional to command potentials. Proportionality factor is (10 ÷ ß) pA/mV.

Model Cell

Description

The PATCH-1U model cell can be used to help with testing and setting up. The pipette is modeled by a 10 MΩ resistor, the cell is modeled by 500 MΩ in parallel with 33 pF (time constant = 16.5 ms), and the patch is modeled by a 10 GΩ resistor. The pipette capacitance is about 4-6 pF.

The PATCH-1U model cell has been made without a switch to change between BATH, PATCH, and CELL. This is because even the best switches have an enormous amount of leakage resistance and capacitance which increases the noise three to five times beyond what you can achieve with a good seal. Instead of switches, three separate plug positions have been provided and you can rotate the model cell into the position required. With this technique the noise contribution of the model cell is still somewhat larger than can be achieved with a good seal, but the increase in noise is less than 50%.

Use

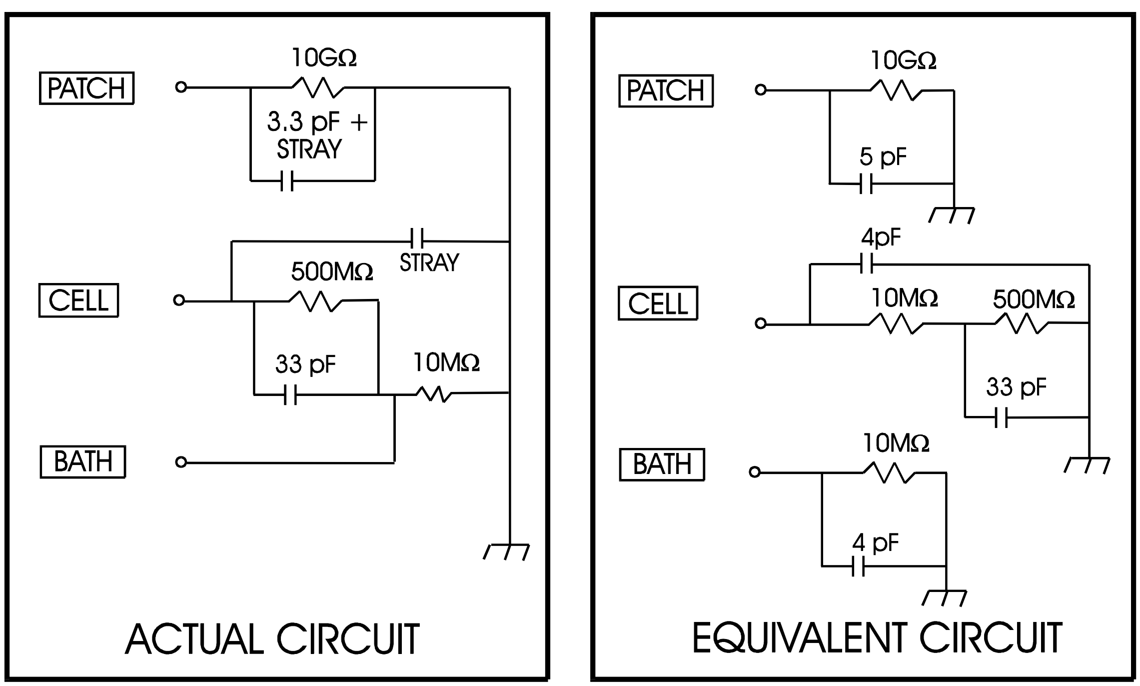
Plug the model cell into the patch-clamp headstage. The gold-plated ground jack on the model cell should be linked to the gold-plated jack on the back of the headstage.

When plugged into the BATH position, the pipette resistor is connected to ground. This is a convenient position for practicing using the junction null controls.

When plugged into the PATCH position, the 10 MΩ pipette resistor is connected to the 10 GΩ patch resistor. In this position a repetitive voltage command can be introduced and the pipette capacitance controls can be set. A small voltage command can be used to check the seal resistance. With a head-stage gain ß = 100, a 20 mV command across a 10 GΩ seal should cause a response current of 2 pA.

When plugged into the CELL position, the 10 MΩ pipette resistor is connected to the cell. Use a ß value of 1 or 0.1. Set up a repetitive voltage command. The capacitance transient should settle with a time constant of about 330 µs (10 MΩ x 33 pF) which corresponds to a 10-90% rise time of about 660 µs. Adjust the WHOLE-CELL CAP. control and/or the SERIES RESISTANCE controls to minimize the current transient. (It is important to carefully set the Fast Capacitance controls. You may need to re-adjust these controls as you use the series resistance. The fast transient can be seen most easily at a filter bandwidth of 5 kHz or more.) In current-clamp mode, the cell voltage should rise exponentially in response to a step current command, with a time constant of about 16 ms.

The PATCH-1U model cell can conveniently be used in conjunction with the tutorial at the front of this manual.



Noise

There are numerous sources of noise in a patch clamp. The two major sources of electronic noise are:

1. The input transistors. The JFET input transistors have been chosen for their low voltage noise and low gate current. The contribution to the total noise from this source is proportional to the input capacitance. Therefore every effort should be made to minimize the added input capacitance from the pipette, the holder, and nearby metal objects.

2. The feedback resistors. Even ideal resistors exhibit thermal noise. In addition, real resistors have excess noise. The origins of this excess noise are not well understood, especially for high-value resistors. The Axopatch-1D headstages use thin-film chip resistors which have been empirically chosen for their combination of low noise and tuneability.

   The thermal noise of the feedback resistor in a current-to-voltage converter is inversely proportional to its value. The 50 GΩ value used for the G = 100 selection was chosen as the highest value that could be used while still maintaining a reasonable current-passing range (200 pA). When the value of the feedback resistor exceeds the seal resistance, the noise is dominated by the noise of the seal resistance.

Oscilloscope Triggering

Typically two timing sources are used during an experiment:

1. The internal oscillator during set-up phases.
2. An external sequencer during recording phases.

It is inconvenient to have to re-cable the external trigger input of the oscilloscope each time the timing sources are swapped. The Axopatch-1D has a facility for mixing (by logical OR'ing) the trigger outputs of the two timing sources, thereby making re-cabling unnecessary.

Suggested Use

Connect the trigger output of the external timing source to the EXT. OSCILLOSCOPE TRIGGER input of the Axopatch-1D. Connect the INT. & EXT. OSCILLOSCOPE TRIGGER output to the EXTERNAL TRIGGER input of the oscilloscope.

Outputs Section

All the controls in the OUTPUTS section only affect the signal on the Scaled Output.

Filter

Twelve -3 dB frequencies in a 1, 2, 5 ratio. These are 20, 50, 100, 200, 500, 1k, 2k, 5k, 10k, 20k, 50k, 100k Hertz. The filter is removed from the output circuit by selecting Bypass.

The filter is a 4-pole Bessel filter. The attenuation of signals and noise above the -3 dB frequency is 80 dB/ decade (24 dB/octave).

The Bessel characteristic is suitable for patch and voltage clamping because it introduces < 1% overshoot.

All lowpass filters slow the rise time of the signal. For filters with < 10% overshoot the 10-90% rise time is:

$$t_r \approx 0.35\,(f_{-3})^{-1}$$

where f_{-3} is the -3 dB frequency in Hertz.

If you use an external filter take care -- some manufacturers specify the -3 dB frequency based on the phase response of the filter instead of its amplitude response, or based on a straight line approximation to the filter characteristics instead of the actual characteristics. You should check your external filter by checking t_r for a step signal applied to its input.

When a signal with 10-90% rise time t_1 is passed through a filter with 10-90% rise time t_2, the rise time of the output signal is approximately:

$$t_r \approx \sqrt{(t_1^2 + t_2^2)}$$

Gain (α)

Eight gain settings in a 1, 2, 5 ratio. These are 0.5, 1, 2, 5, 10, 20, 50, 100. Note that the Bessel filter precedes the gain amplifier.

Frequency and Gain Setting Outputs

Analog outputs are provided that correspond to the frequency and gain settings.

Frequency (Hz)	20	50	100	200	500	1k	2k	5k	10k	20k	50k	100k
Telegraph Output (V)	0.4	0.8	1.2	1.6	2.0	2.4	2.8	3.2	3.6	4.0	4.4	4.8

These values are negative if Bypass is selected

Gain (α)	0.5	1	2	5	10	20	50	100
Telegraph Output (V)	0.4	0.8	1.2	1.6	2.0	2.4	2.8	3.2

These values are negative if ß = 100.

These values have been chosen to fit in the range 0 - 5 V so that they can be read on ADC channels of most computers.

Output Selection

The signal to be processed by the OUTPUT section is chosen on the OUTPUT SELECT switch.

1. V_m: The output signal is V_m X 10α (where α is the value of the GAIN setting).
2. I: The output signal is ßα mV/pA (where ß is the headstage gain).
3. EXT: The output signal is α times the signal connected to the EXT. SIGNAL TO OUTPUT SECTION input.

The selected signal (without any output processing) is also the input to the audio monitor.

Leak Subtraction

Typically the passive membrane response to a voltage step consists of a transient and a steady-state component. It is often helpful to subtract these from the output so that only active responses are observed.

The transient component is eliminated by using the CAPACITANCE COMPENSATION controls discussed in the section of that name. The steady-state component is eliminated by the LEAK SUBTRACTION control.

The normal procedure is to repetitively apply a small voltage step that will only activate passive currents (*e.g.*, 10 mV hyperpolarization). Observe I. After eliminating the capacitance transient, turn the LEAK SUBTRACTION potentiometer clockwise from ∞ until the current step disappears. Since both the Capacitance Compensation and Leak Subtraction controls are driven by the command voltage, the passive responses remain eliminated for all polarities and magnitudes of command.

Note that the OFF position of the LEAK SUBTRACTION control is "∞", *i.e.*, the leakage resistance through the membrane (or seal) is ∞. The maximum position is 10ß, corresponding to 1 MΩ, 10 MΩ and 1 GΩ leaks for ß = 0.1, 1 and 100, respectively. If the LEAK SUBTRACTION control is not in use, turn it firmly counterclockwise so that it clicks OFF.

If the passive membrane responses are too complex to be eliminated by the built-in controls, an externally derived estimate (*e.g.*, by computer or an analog membrane model) of the passive currents can be connected to the Leak Subtraction input and subtracted from the output. The input sensitivity is $ß^{-1}$ pA/mV. The input impedance is 20 kΩ. The command signal -20 V_{CMND} is provided for use by the external circuit.

Auto Output Zero

The purpose of this control is to zero the output. That is, remove the DC voltage.

Zeroing is performed rapidly when the RESET button is pressed. Thereafter, the output is DC coupled with an initial DC value of zero.

The toggle switch must be in the ACTIVE position for output zeroing to work. If the switch is moved to the OFF position, the Scaled Output will return to the DC value it would have had if the Auto Output Zero had never been used. An LED lights when the switch is in the ACTIVE position.

The Auto Output Zero only affects the signal on the Scaled Output. No other input or outputs are affected.

Distortion-free AC Coupling

Reset can also be activated by the low-to-high transition of a logic pulse applied to the Auto Output Zero Reset input. Resetting takes 50 µs.

To achieve distortion-free AC coupling, trigger RESET at the start of each oscilloscope sweep or data input sweep.

Principle of Operation

The unamplified signal is converted into a 12-bit digital representation each time RESET is activated. This digital word drives a 12-bit digital-to-analog converter to produce a new analog signal of the same magnitude as the input signal. This process constitutes a digital sample-and-hold.

The sampled analog signal is subtracted from the time-varying input signal. The difference of the two is then passed to the Gain amplifier. Immediately after Reset this difference signal is zero. Thereafter, the difference signal is the full-bandwidth input signal, but with the DC content effectively removed.

Because the sample of the signal is held digitally, there is none of the drift associated with analog sample-and-holds. However, a different problem arises: because the sample is digital, there is a quantization error. That is, the signal is only sampled to within 10 mV accuracy. This quantization error is normally too small to be noticed on the oscilloscope screen if the vertical oscilloscope display is set to display the full signal.

Output Calibration

An output calibration signal can be superimposed on 10 V_m, I and the Scaled Output.

Internal

A +100 mV internally generated calibration voltage can be activated by switching the front-panel switch to the CONT. position. With the switch in the GATE SIGNAL position, the +100 mV calibration voltage is off unless a logic high level is applied to the rear-panel 100 mV CAL. GATE input. The OFF position disables the external logic command.

On the Scaled Output, the calibration voltage is multiplied by the Gain setting.

External

A calibration voltage proportional to an external signal can be added to the outputs by applying the external signal to the EXT. CAL. SIGNAL input while the front-panel switch is in the GATE position. The voltage appearing on the outputs will be 10 mV per volt of external signal.

Suggested Use

When recording onto a tape or chart recorder, at the start of the recording toggle several times from OFF to CONT. to record a 100 mV reference onto the tape or chart.

When recording directly to a computer, use the computer to gate and record the 100 mV calibration whenever you reconfigure the gain of the recording pathway.

Pipettes

To take maximal advantage of the electronics in any patch clamp, it is imperative that care be given to the construction and use of pipettes. Both the noise and the dynamic performance of the patch-clamp measurement depend on pipette quality. Compensation of capacity transients and series resistance can only be done optimally when pipette technology is optimal.

Glasses

There are about 30 types of electrically acceptable glasses available commercially for patch clamping. We have experience with 25 of these. All are capable of producing gigohm seals to membranes, but three of the glasses are notable in their performance.

Corning #7052 Kovar Sealing glass seals exceptionally well to a variety of cells when it is pulled and firepolished properly. It has the electrical properties of "hard" glasses but thermal properties more similar to soda lime glass than to hard glass.

Corning #7040 Kovar Sealing Glass has similar sealing and thermal properties to #7052 but is better electrically and can result in lower-noise recordings.

Corning #8161 has produced the lowest-noise recordings of any glass we have tried. It seals well but pulls at a considerably lower temperature than even soda lime glasses. Its noise is as good as, or better than, Corning #1723 aluminosilicate glass, but it does not require the high heater currents necessary for #1723. It is the most versatile of glasses we have tried since it can be used for low-noise, low-loss patch electrodes and whole-cell electrodes.

All of these glasses can be obtained from specialty glass houses like:

Clark Electromedical Instruments
P.O. Box 8, Pangbourne, Reading, RG8 7HU, UK; Phone: (073) 573-8888.

Garner Glass
177 S. Indian Hill Rd, Claremont, CA 91711, USA; Phone: (909) 624-5071,
Fax: (909) 624-7212.

Jencons Scientific
Cherrycourt Way Industrial Estate, Stanbridge Road, Leighton Buzzard, Bedfordshire, LV7 8UA, UK, Phone: (0525) 372010.

Sutter Instrument Company
40 Leveroni Court, Navato, CA 94949, USA; Phone: (415) 883-0128.

Pulling

Patch or whole-cell pipettes generally require a two stage pull (see Hamill *et. al.*, 1981, or Rae and Levis, 1984, for discussion). This means that the glass is pulled to an hour glass shape in the first pull. After the first pull, the hour glass is repositioned into the heater coil and pulled a second time until the two halves separate. Several pullers are commercially available to facilitate this operation (*e.g.*, Sutter Instrument Company, see address above). The pipette requirements for patch clamping and whole-cell clamping are somewhat different. Pipettes for whole-cell clamping should be pulled to minimize access resistance, so low resistance electrodes with blunt tips should be constructed. This is best done with #8161 glass. Because of the thermal properties of this glass, it is possible to pull tips that are essentially broken and ragged and of very large size (> 25 µm), then fire polish these to an almost bullet shape to produce a low, nearly lumped access resistance. Such pipettes will facilitate the compensation of series resistance and allow the highest-bandwidth, lowest-noise whole-cell recordings.

Coating

All pipette glasses require coating with a hydrophobic substance like Sylgard #184 (Dow Corning, Inc.). The hydrophobic coating keeps the bath fluid from creeping up the outer glass wall and forming a distributed noise source. In addition, the electrical properties of the Sylgard are better than any of the glasses we have tried, and therefore the coating improves the basic electrical properties of the wall. It particularly reduces the wall capacitance and thus reduces noise, making capacity compensation more easily accomplished. For best results, the Sylgard should extend as close to the tip as possible, but for good electrical glasses like those described above, painting to within 100 microns or so from the tip is sufficient for good results (see Hamill *et. al.*, 1981, and Rae and Levis, 1984, for a detailed discussion). The Sylgard requires heat curing with, for example, a heat gun or some other source.

Filling

Pipettes are filled in two stages. The tip is filled by applying suction to the back of the pipette while the tip is immersed in filtered filling solution. The remainder is back filled through an appropriate-diameter syringe needle using a small syringe and syringe filter. It is important for noise considerations to keep the fluid level in the pipette as low as possible, usually just sufficient to cover the tip of the electrode in the pipette. It is also important to be sure that no fluid exists in the upper part of the pipette which might get into the pipette holder. A gentle stream of gas to dry solution droplets near the top of the pipette is useful.

Power Supply Glitches

The Axopatch-1D has been designed to minimize the effects of power supply transients (glitches). This is achieved by:

1. taking the incoming power through a radio-frequency interference (RFI) filter, and
2. capacitively isolating the transformer primaries and secondaries.

Nevertheless, some power-supply glitches do get through. These can cause transients to appear on the voltage and current outputs which may corrupt high-sensitivity recordings.

The only completely effective way to gain immunity from mains glitches is to eliminate them at the source. Most glitches are due to the switching on and off of other equipment and lights on the same power-supply circuit. Precautions to be taken include:

1. Avoid switching equipment and lights on or off while recordings are being made.
2. Water baths, heaters, coolers, etc. should operate from zero-crossing relays.
3. RFI filters should be installed in glitch-producing equipment.

In most circumstances, occasional transients on the outputs are inconsequential and therefore no precautions have to be taken.

Power Supply Voltage Selection and Fuse Changing

Supply Voltage

The Axopatch-1D can work from all international supply voltages. The two input ranges are:

1. 115 V : For 100 V~ to 125 V~ operation.
2. 230 V : For 200 V~ to 250 V~ operation.

To change the supply voltage setting:

1. Disconnect the power cord.
2. Remove the top cover.
3. Locate the slide switch labeled "S2" at the back of the power-supply board. The power-supply board is the small horizontal board in the left side of the instrument.
4. For 115 V operation slide S2 to the left towards the label "115". For 230 V operation slide S2 to the right towards the label "230".
5. Replace the top cover.
6. Re-connect the power cord.

7. Mark the new operating voltage on the identification plate on the rear of the instrument.

Changing the Fuse

The Axopatch-1D uses a 0.5 A 250 V slow acting 5 x 20 mm fuse on both voltage ranges. Before changing the fuse investigate the reason for its failure.

To change the fuse:

1. **Disconnect the power cord.**
2. Use a screwdriver or something similar to lever out the fuse holder.
3. Discard the fuse from the active slot (*i.e.*, the slot which places the fuse closest to the inside of the instrument).
4. Shift the spare fuse from the spare slot (*i.e.*, the slot which places the fuse towards the outside of the instrument) to the active slot.
5. Reconnect the power cord.

RMS Noise Display

Purpose

The RMS noise is a sensitive measure of the cleanliness and quality of the pipette, the seal, and the holder.

RMS Meter

You must choose whether to display the RMS Noise of the current (I) or of an external signal connected on the rear panel to the input RMS Meter connector. For I, the display is in pA or nA and is automatically scaled to suit the headstage gain. For EXT. the display is in mV.

Bandwidth

When I is selected the RMS current-noise measurement bandwidth is 30 Hz to 5 kHz. The lower limit is determined by a one-pole highpass filter. The upper limit is determined by a four-pole lowpass Butterworth filter.

When EXT. is selected, the lower limit of the noise measurement bandwidth is 30 Hz. The upper limit is the bandwidth of the external signal. For example, if you wanted to display the current noise in the same 1 kHz bandwidth that you are using for recording, you would:

1. Set the BESSEL FILTER to 1 kHz.
2. Connect the SCALED OUTPUT signal to the INPUT TO RMS METER connector.
3. Select EXT. on the RMS METER SELECTOR switch.

As you change the filter setting, the RMS Meter will display the noise in the selected bandwidth. In this example, you will need to divide the RMS display by the Gain (α).

Expected Values

With no holder connected, and with the headstage input carefully shielded, the RMS readings (I selected) should be:

< 0.15 pA	for	ß = 100
< 0.6 pA	for	ß = 1
< 0.002 nA	for	ß = 0.1

Seal Formation

This section provides information on how to recognize electrically the formation of the seal. The methodology for forming a seal is beyond the scope of this manual. We recommend that you read the Single-Channel Recording book edited by Sakmann and Neher (1983) for this information.

There are two clear indications that a seal has been formed:

1. The peak-to-peak current noise goes down dramatically.
2. The magnitude of the test pulse goes down dramatically. The test pulse would normally be generated by using the R_{SEAL} Test facility driven from the internal oscillator. Alternatively, you could use the Step Command or an external command.

The amplitude of the current response depends on the combined resistance of the pipette plus seal (R_{tot}) and the headstage gain (ß):

$$I = V_p / R_{tot}$$

If you are using a 0.2 mV test pulse (R_{SEAL}), ß = 100, then for various values of R_{tot} the current amplitude will be:

R_{tot} =	1 MΩ, I	= 200 pA
R_{tot} =	10 MΩ, I	= 20 pA
R_{tot} =	100 MΩ, I	= 2 pA

After seal formation, switch to a 20 mV test pulse:

R_{tot} = 1 GΩ, I = 20 pA

R_{tot} = 10 GΩ, I = 2 pA

Series Resistance

In patch clamping, the term "Series Resistance" refers to the pipette resistance plus any other access resistances to the patch or cell.

The Series Resistance Compensation controls (SERIES RESISTANCE, % COMPENSATION, WHOLE-CELL CAP.) are only active if ß = 1 or 0.1.

Absolute Value

The absolute value of the series resistance shows on the SERIES RESISTANCE dial after the whole-cell current transient has been eliminated. This procedure is described in the *Capacitance Compensation* section.

% Compensation

Eliminating the current transient by setting the Series Resistance and Whole-Cell Cap. does not improve the speed of clamping the cell. Using an independent microelectrode in the cell would show that the cell charging rate is not affected by this procedure. This is because it is still the pipette plus cell that is clamped; thus the cell membrane must passively charge through the pipette series resistance.

The reason for eliminating the current transient with the SERIES RESISTANCE and WHOLE-CELL CAP. controls is that the series-resistance correction circuitry, when invoked, could not cope with these large transient signals.

Once the transient is eliminated, the % COMPENSATION control is advanced as far as possible to correct for the effects of the series resistance. This correction is twofold:

1. The voltage error due to current flow through the pipette is reduced by the indicated percentage.

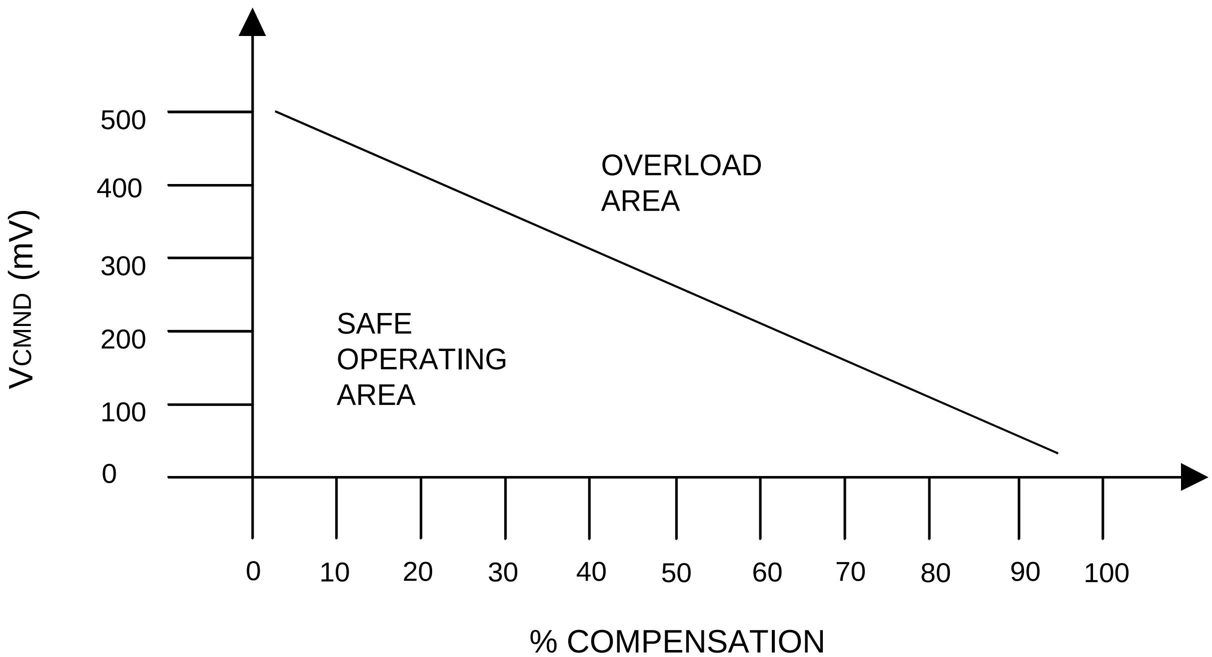
2. The membrane charging time is reduced by the indicated percentage.

As the % COMPENSATION control is advanced, the current becomes noisier. A transient will appear out of the noise. Fine adjustments to the FAST MAG. and WHOLE-CELL CAP. controls can minimize the size of this transient.

As the % Compensation is advanced, the effective resistance of the electrode is reduced. As a result, the time constant for charging the membrane is similarly reduced. If nothing was done about this, the whole-cell compensation controls would become out of balance because they were set at the original membrane charging time constant. Special circuit designs are used so that the whole-cell compensation is automatically adjusted to follow the reduction in the membrane charging time constant.

Saturation

The series-resistance correction circuitry will saturate when the % Compensation and the voltage command exceed certain limits as shown in the graph below. The saturation will be obvious as odd, nonlinear distortions of the current transient.



Oscillations

One of the practical problems when using series-resistance compensation is that there is a great risk of oscillation. This is because series-resistance compensation is a form of positive feedback. The main cause of oscillations is that the circuitry cannot distinguish current that flows down the pipette and into the cell from current that flows through the stray capacitance of the pipette into the bath. The current that flows through the electrode resistance into the cell is the current that it is intended to compensate. The compensation circuitry also tries to compensate for the current into the pipette capacitance. However, in this case there is no resistance component to compensate and the compensation circuit will oscillate as soon as the % Compensation is advanced.

The tendency to oscillate depends therefore on the relative magnitude of the pipette resistance to the pipette capacitance and the degree of compensation of the pipette capacitance.

Using Lag to Prevent Oscillations

The tendency to oscillate can be reduced by limiting the bandwidth of the positive-feedback circuit. This is the function of the LAG control. Normally you should try to get the best series-resistance compensation that you can by carefully setting the Fast Capacitance Compensation controls prior to advancing the % Compensation. If, however, oscillations begin before you have reached an adequate level of compensation, advance the LAG control. Only advance the Lag as far as you need to maintain stability because the more it is used the less compensation there is of high-frequency currents.

Suggested Use

Apply a repetitive step command. Eliminate the capacitance-charging current transient by setting the SERIES RESISTANCE and WHOLE-CELL CAP. controls as described in the *Capacitance Compensation* section. Advance the % COMPENSATION, with the LAG on minimum. It is normal for the current noise to increase because compensating for the series resistance actually increases the recording bandwidth.

As you increase the % COMPENSATION there will likely be a tendency towards oscillation. This can usually be removed by carefully readjusting the FAST MAG. control. If you iteratively increase the % COMPENSATION and fine tune the FAST MAG., you will often be able to achieve 80% compensation or more. It is easier to set the Fast Mag. Compensation if you set the -3 dB filter frequency to 10 kHz or more.

Using the LAG control can increase the maximum achievable % Compensation. The trade-off is that it reduces the compensation bandwidth.

ß = 0.1 for Large Cells

The standard ß = 1 headstage is not suitable for large cells with membrane capacitances greater than 100 pF. To deal with larger capacitances it is not practical to simply increase C2 inside the headstage because this linearly increases the high-frequency noise.

The alternative approach used in the Axopatch-1D is to use a ß = 0.1 headstage. This has a smaller feedback resistor (R2 = 50 MΩ) and no C2 capacitor.

To use the ß = 0.1 headstage on a large cell, no attempt is made to eliminate the capacitance-charging transient by using the WHOLE-CELL CAPACITANCE and SERIES RESISTANCE controls. The series resistance of the pipette must be estimated by other means (*e.g.*, by the balancing technique described in the *Current Clamp* section) and set on the SERIES RESISTANCE control. The % COMPENSATION control can then be used normally. The WHOLE-CELL CAPACITANCE COMPENSATION control should be set at zero.

Limitations

Series resistance compensation is an attempt to electronically reduce the effect of the pipette resistance. Because of practical limitations, it is never perfect. Even if 100% compensation could be used with stability, this would only apply to DC and medium-speed currents. High-speed currents would be corrected by less than 100%.

For best results, the cell input resistance should be many times the pipette series resistance. This is normally the case for cells at rest carrying small drug-activated or synaptic currents. However, during voltage activation the cell input resistance could fall a hundredfold or more to values similar to or less than the series resistance. In these cases it is probable that:

1. There will be a significant error due to the voltage drop across the pipette. This error is subtle because the patch clamp only records the combined voltage drop across the pipette and the cell.

2. The setting of the SERIES RESISTANCE and WHOLE-CELL CAP. Compensation controls will become erroneous because they were set based on the time constant to charge the membrane capacitance before the change in the membrane resistance. Since this time constant depends on the parallel value of the membrane resistance and the electrode series resistance, this error could become substantial. The effect will be a larger transient at voltage levels that activate the fall of membrane resistance.

If the cell input resistance becomes comparable to or less than the pipette resistance, the whole-cell patch technique will probably not work. In this case, it would be more accurate to use a discontinuous (chopped) single-electrode voltage clamp such as the Axoclamp.

Ten-Turn Potentiometers

The ten-turn potentiometers used in the Axopatch-1D are high-quality wirewound types.

An inherent problem of wirewound potentiometers is that the wire elements tend to oxidize. This condition is easily cured. If a potentiometer becomes noisy, the potentiometer manufacturer recommends rapidly spinning the knob 20-30 times between full clockwise and full counterclockwise. This clears the oxide off the element and restores noise-free operation.

Trouble Shooting

It has been our experience at Axon Instruments that the majority of troubles reported to us have been caused by faulty equipment connected to our instruments.

If you have a problem, please disconnect **all** instruments connected to the Axopatch-1D except for the headstage. Work completely through the *Functional Checkout* section of the **GETTING UP AND RUNNING - A TUTORIAL** chapter. This can often uncover a problem that is in your set up. If the problem persists, please call us for assistance.

Zap

In order to go from cell-attached patch clamping to whole-cell patch clamping it is necessary to rupture the patch. This is normally done by carefully controlled suction.

Another, easier to apply, technique for rupturing the patch is Zap. Zap works by applying a large voltage (1.5 V DC) to the patch for a controlled duration. This often causes dielectric breakdown of the membrane.

Suggested Use

Select ß = 1 or ß = 0.1. Apply a repetitive test command (*e.g.,* R_{SEAL} Test). Start with DURATION = 0.1 ms. Press TRIG. to zap the membrane. Successful zapping is accompanied by an increase in the current noise and by large capacitance-charging current transients in response to the test command. Use the briefest zap that will rupture the membrane. Too long a zap could cause the seal resistance to deteriorate.

SPECIFICATIONS

CV-4 Headstage

Construction: All critical components are in a sealed hybrid.

Configuration: High-speed low-noise current-to-voltage converter.

Headstage Gain(ß): ß refers to output in mV/pA. Proportional to feedback resistor (R_f). Scaling is used to simplify ß values to powers of 10.

ß	=	100	for	R_f =	50	GΩ
ß	=	1	for	R_f =	500	MΩ
ß	=	0.1	for	R_f =	50	MΩ

Please note carefully the meaning of ß.
It will be used frequently in these specifications.

Feedback Resistor Selection: FET switches in hybrid enable remote selection of either of two values. ß = 100 and ß = 1 are standard. Value selected must be available in headstage for correct operation.

Tuning: Tuning circuits to idealize response of feedback resistors are contained in a small box in headstage cable. Tuning automatically switches to suit feedback resistor selected.

Options: CV-4-1/100U is standard
CV-4-0.1/100U for large cells requiring clamp currents greater than 20 nA
CV-4B-0.1/100U for bilayers

Electrode-Capacitance-Compensation Injection Capacitor Value: 0.5 pF

Whole-Cell-Capacitance-Compensation Injection Capacitor Values:

None	for	ß	=	100
5 pF	for	ß	=	1
None	for	ß	=	0.1

Case: Case connected to ground. Case jack mates to 2 mm plugs.

Bandwidth: Test signal applied via capacitor direct to input; no holder or electrode. Typical -3 dB frequency:

20 kHz	for	ß	=	100	(6 kHz with a CV-4B)
80 kHz	for	ß	=	1	
80 kHz	for	ß	=	0.1	

Noise: Measured from DC to stated bandwidth, 8-pole Bessel filter, ß = 100, guaranteed maximums.

Without holder:	DC-1	kHz;	0.04	pA	RMS
	DC-3	kHz;	0.1	pA	RMS
	DC-10	kHz;	0.3	pA	RMS
With holder:	DC-10	kHz;	0.35	pA	RMS

Headstage Connectors: Socket for ground output is 0.08” (2 mm) diameter. Input socket is threaded Teflon and accepts a 0.04” (1 mm) pin.

Pipette Holder: HL-U holders mate to the threaded Teflon input connector of the CV headstage. Post for suction tubing is 1 mm OD. The HL-U holder accecpts glass 1.0-1.7 mm OD. Supplied with silver wire. Optional HLR-U right-angle adapter and HLB-U BNC adapter are available..

Current Clamp

The speed in current clamp depends on the cell and the electrode. The following time constants for a step current were measured in several model cells:

R_m	C_m	R_e	Current step time constant
2 GΩ	10 pF	10 MΩ	100 µs
500 MΩ	33 pF	10 MΩ	100 µs

Pipette Holder

HL-U holder mates to threaded Teflon input connector of the CV headstage. Post for suction tubing is 1 mm OD. HL-U holder accepts glass 1.0-1.7 mm OD. Supplied with silver wire. Optional HLR-U right-angle adapter and HLB-U BNC adapter are available.

Capacitance Compensation

1. **Fast τ:** 0.2-5 µs
   Fast Magnitude: 0-10 pF
   Slow τ: 0.1-10 ms
   Slow Magnitude: 0-1 pF

   These controls are used to charge electrode capacitance. In V-CLAMP mode they speed the response to command voltages. In I-CLAMP mode they act as a negative capacitance.

2. **Whole-Cell Capacitance:** 0.3-100 pF
   Series Resistance: 0-100 ß MΩ

   These controls are used to charge membrane capacitance in whole-cell voltage clamp. For ß = 0.1, whole-cell capacitance is not operative. In I-CLAMP mode Series Resistance compensates electrode IR voltage drop. Whole-Cell Capacitance Compensation is not operative. Controls are not operative for ß = 100.

Series Resistance Compensation

% Compensation: OFF, 0-100%. Full scale magnitude determined by Series Resistance setting.
Lag: 1-100 µs. Cuts high-frequency response of compensation circuit.

Mode

V-CLAMP: Pipette voltage is clamped.
I = 0: Pipette current is clamped to zero.
I-Clamp: Pipette current is clamped. Response to voltage commands is 10 ÷ ß pA/mV.

Command Potentials

Step Command: ±199.9 mV max. Set on thumbwheel switch. Activated by HI control signal on Step Activate input, by front-panel switch or by internal oscillator.

Speed Test: Injects current into headstage input via compensation capacitor. Approximately 100 pA command at internal oscillator frequency or external signal at 100 pA/V for ß = 100. About 10 times these values for ß = 1. An integrator preserves waveform type. Bandwidth: 0.3 Hz to 500 kHz.

R_{SEAL} Test: 0.2 mV or 20 mV command at internal oscillator frequency.

External Command: Two sensitivities; 20 mV/V or 1 mV/V. Input impedance: 10 kΩ

Holding Potential: ±200 mV. Ten-turn potentiometer with dial. Polarity switch.

Oscillator: Frequencies: 1, 10, 100 Hz
Accuracy: 10%
Duty cycle: 33%

Zap

Amplitude: +1.5 V at pipette. Not adjustable.

Duration: 0.1-10 ms. Log potentiometer. Triggered by front-panel pushbutton.

Junction Null

Manual: ±200 mV. Ten-turn pot.

Auto: Nulling potential adjusts to maintain zero pipette current. Approximately 100 ms time constant in TRACK mode. When HOLD mode is selected, value is sampled and held indefinitely. Reset button for fast nulling. LED flashes in HOLD, shines continuously in Track.

Audio Monitor

Pitch is proportional to signal selected on OUTPUT SELECT switch. Internal speaker is bypassed when earphone is plugged in.

RMS Noise

3.5 digit meter displays RMS current noise in pA for ß = 100 and ß = 1, in nA for ß = 0.1, or RMS noise of external signal in mV. Measurement bandwidth is 30 Hz to 5 kHz. Upper -3 dB frequency is set by 4-pole Butterworth filter. Analog value appears on RMS VALUE output.

Oscilloscope Trigger

Trigger from external source is connected to EXT. OSCILLOSCOPE TRIGGER input. Logically ORed with internal oscillator trigger. Combined trigger goes to INT. & EXT. OSCILLOSCOPE TRIGGER output.

Outputs

I: Pipette current. ß mV/pA

$10V_m$: Membrane potential at x10 gain. Bath and junction potentials removed.

$-20V_{CMND}$: Command potential at x20 gain. Inverted.

$20V_{BATH}$: Bath potential at x20 gain.

$10V_p$: Pipette potential at x10 gain.

Scaled Output: I or $10V_m$ scaled by output control settings.

Output Offset: Voltage removed from Scaled Output by Auto Output Zero circuit.

Output Controls

Output Gain(α): 8 values from 0.5-100. Affects Scaled Output only. Selected value sets analog voltage on Gain Setting output for reading by computer. 0.4 V / increment starting at 0.4 V for α=0.5, inverted for ß = 100.

-3 dB Frequency: 4-pole lowpass Bessel filter. 12 frequencies from 20 Hz to 100 kHz. Affects Scaled Output only. Selected value sets an analog voltage on Frequency setting output. 0.4 V / increment starting at 0.4 V for 20 Hz, inverted for filter on Bypass. Filter precedes gain amplifier.

Leak Subtraction:

Internal: Signal proportional to command subtracted from current record. Range: 10ß MΩ to ∞.

External: Subtraction proportional to applied voltage, $ß^{-1}$ pA/mV.

Auto Output Zero: After Reset, zeros the DC voltage on Scaled Output. Range is ±10 V for α = 0.5-5, ±5 V for α = 10, falling to ±0.5 V for α = 100. Reset by HI control signal on Reset AUTO OUTPUT ZERO input or by pressing pushbutton switch. Reset time is 1 ms.

Main Meter

3.5 digit meter displays Track potential (V_{TRACK}) membrane potential (V_m) and bath potential (V_{BATH}) in mV, or current (I) in pA.

Output Calibration

Internal: 100 mV added to I, 10V_m and Scaled Output when activated by HI control signal on 100 mV Cal Activate input or by front-panel switch.

External: Signal added to outputs is proportional to applied voltage; 10 mV/V.

Bath Potential Subtraction

BH-1 headstage may be used to record bath potential at x20 gain, 1 kHz bandwidth. If bath potential is measured, it is used as reference potential. If not, 0 V is used as reference potential.

Grounding

Signal ground is isolated from chassis and power ground.

Control Inputs

Above 3 V accepted as logic HI. Below 2 V accepted as logic LO. Inputs protected to ±15 V.

Model Cell

The PATCH-1U model cell emulates three experimental conditions:

Bath:	10 MΩ	electrode resistor to ground; 4 pF electrode capacitance.
Cell:	10 MΩ	electrode resistor connected to a 500 MΩ // 33 pF cell. 4 pF electrode capacitance.
Patch:	10 MΩ	electrode connected to a 10 GΩ patch. 5 pF electrode capacitance.

Headstage Dimensions

Case is 2.25 x 1.14 x 0.87" (57.2 x 29.0 x 22.1 mm). Mounting rod is 4" (102 mm) long. Available mounting rod diameters (D) are 1/4, 5/16 or 3/8" (6.3, 7.9 or 9.5 mm). Specify required mounting-rod diameter with order. A removable polycarbonate mounting plate 2.45 x 1.94 x 0.25" (62 x 49 x 6.3 mm) is supplied. Cable length is 10 feet (3 m).

Case Dimensions

7" (177 mm) high, 19" (483 mm) wide, 12.5" (317 mm) deep. Mounts in standard 19" rack. Handles included. Net weight 18 lbs (8 kgs).

Supply Requirements

Line Voltage: 100-125 V~ or 200-250 V~. User selectable by an internal switch.

Line Frequency: 50-60 Hz.

Power: 20 W

Fuse: 0.5 A slow. 5 x 20 mm.

Line Filter: RFI filter is included.

Line cord: Shielded line cord is provided.

Accessories Provided

Theory and Operation Manual
One HL-U pipette holder
PATCH-1U model cell
Spare fuse

Ordering Information

When ordering please specify diameter (D) of headstage mounting rod. D = 5/16" (7.9 mm) is default value.

Warranty

12 months parts and labor from date of receipt.

Service

Service is available at the factory.

For further information call us. A factory expert will be pleased to answer your technical and ordering inquiries.

SERVICE

General

The Axopatch is designed for measuring pA currents at bandwidth of tens of kHz.

To assist you in interpreting the circuit schematics, we list here the meaning of some widely used signals:

G100	High when headstage gain (ß) is 100
G1	High when ß = 1
G01	High when ß = 0.1
I2A	Current output of headstage
I2	Headstage current after offset correction
I	Headstage current scaled to 10^n pA/mV
+VREF	+5 V stable reference
-VREF	-5 V stable reference
VCLAMP	High when V-CLAMP mode selected

Headstage Drive (refer to A-2)

The command voltage to the positive input of the headstage current-to-voltage convertor is called VP. To minimize wideband noise, commands are generated at x20 gain, then resistively attenuated.

VP derives from the output of U32 or U35 depending on the position of relay K7. In V-CLAMP mode the summing amplifier U32 is selected. In I-CLAMP mode or I = 0 mode the current-clamp integrator U35 is selected. Opening switch U30A at the input of U35 during I = 0 mode ensures that U35 clamps the pipette current to zero irrespective of the command potentials.

Series resistance compensation is added into VP by relay K6.

The current output of the headstage is I2A. This is scaled by x2 in amplifier U27 to generate the current I seen by the user. I0 and I1 are the current measured before and after, respectively, the first stage in the headstage boost box.

Before proceeding to the rest of the circuitry, if the headstage gain is high (ß = 100), I2A goes through an offset-correction amplifier U26. This feature is not functional in the current model, thus the input to U25B is grounded.

The Zap circuit (U30B and U33B) applies a 1.4 V pulse to VP for a duration set on the potentiometer.

Correction Potentials (refer to A-3, A-6 to A-16)

The signal -20VCRTN is a correction signal containing the manual junction null, the automatic junction null and the bath potential.

The bath potential 20VB comes from the BH-1 headstage. A link in the BH-1 headstage connector activates relay K4.

The manual junction null is set on a potentiometer.

Control of the automatic junction null is by gates U13 and U14. These control circuits are passed to the plug-in Track board (3430-012) through connector J3.

The Track/Hold circuitry is shown on pages A-6 to A-16.

Comparator U22 determines whether the 16-bit counter U14, U12, U13, U20 counts up or down, depending on whether I2 is greater than or less than zero. The counter outputs drive the 16-bit D/A converter U19. Its output goes to the summing amplifier U10 on the main board (3430-011) and drives I2 back towards zero.

The full-wave rectifier U24, U25 drives the voltage-controlled oscillator U23. The frequency of the clock output is thus proportional to absolute magnitude to the current. The actual clock going to the 16-bit counter to drive the D/A is selected in the U16, U21 network.

Command Potentials (refer to A-4, A-16)

The command generating circuitry is located on the plug-in Track board (3430-012). It connects to the main board (3435-011) through connector J4.

The primary command potential is the thumbwheel switch assembly 3435-004. Its output is a DC voltage which is fed through to the rest of the circuitry when switch U4 is activated. Activating U4 depends on the selection on the 4-pole OSC/CONT/GATE/OFF switch.

A logic-level oscillator, U10, can be used for activating U4 and various other circuits.

The Holding Position, the External Command, the Step (thumbwheel) Command and the R_{SEAL} command are all summed in U8 before being sent to the headstage drive circuitry.

Electrode Capacitance Compensation (refer to A-5)

To compensate the pipette capacitance, a filtered version of VP is injected into the headstage input through capacitor C1 located in the headstage. Two time constants and magnitude controls are provided. The two filtered signals are summed in U20.

To test the headstage performance, a known current waveform must be injected through C1. Since the current through C1 is the derivative of the voltage across it, U17 is used to integrate the command voltage before applying it to C1.

Series Resistance Correction and Whole-Cell Compensation (refer to A-6)

To compensate for the voltage drop across the pipette it is necessary to feed a voltage back to the headstage command input (VP) proportional to the pipette current (I2). U1 provides some high-frequency attenuation to I2. U2 and U3 are used for scaling. The signal coming into U3 from U6 lets the series resistance correction circuitry know how much pipette current is being supplied by the whole-cell compensation circuitry. When ß = 0.1, the whole-cell compensation circuit is disabled. This is achieved by grounding the signal from U6 through K1A.

In current-clamp mode, the output 5VRS is proportional to the current command $-20V_{CMND}$. In this mode 5VRS is not connected to VP. Instead it is used by the membrane potential measurement circuit shown on page A-10.

To compensate the cell membrane capacitance, a filtered version of $20V_{CMND}$ is injected into the pipette through capacitor C2 located in the headstage. This capacitor is not installed in a CV-4-0.1/100U headstage.

The filter time constant is proportional to the series resistance of the pipette (set on the SERIES RESISTANCE potentiometer) and the membrane capacitance (set on the WHOLE-CELL CAP. potentiometer). Amplifier U4, together with C3, forms a variable capacitor. Again, for the CV-4-0.1/100U only, this signal does not get injected into pipette.

The output of amplifier U6 is proportional to the C2 current. When the % of series resistance compensation is increased the C2 current must be sped up proportionately. This is automatically achieved by feeding the output of U6 into U7, through the % COMP. potentiometer. This potentiometer is ganged to the % COMP. potentiometer in the Series Resistance Correction circuit.

Filter (refer to A-7)

The filter is a variable-cutoff frequency 4-pole Bessel. The 4 identical variable resistors RA, RB, RC and RD are set on a 12-position switch.

A voltage proportional to the frequency setting is generated with a fifth switch pole. This Frequency Setting output is positive if the filter is active, but negative if the filter is bypassed.

Gain Amplifier and Output Zero (refer to A-8)

Variable gain is provided by U71. A voltage proportional to the gain setting is generated with a second pole. This Gain Setting output is positive for ß = 0.1 or ß = 1, but negative if ß = 100.

The rest of the circuitry is for automatic zeroing (*i.e.*, DC removal). The input signal FILTOUT is filtered in U65A, then digitized in the 12-bit A/D U66 each time a Reset pulse is generated. The stored 12-bit word is reconverted to analog in U63 and U64. This value is subtracted from the unfiltered input signal in U65B.

Assorted (refer to A-9, A-10, A-11)

Headstage gain: This logic circuitry is used for switching, in the main unit and in the headstage.

Membrane potential: In V-CLAMP mode, the membrane potential (VM) is proportional to $20V_{CMND}$. In I-CLAMP mode, VM is proportional to 20VCC, the output of U35 on page A-2 and A18 less the correction potentials (reference A-3) and the voltage drop across the pipette series resistance (A-6).

Output select: One of I2, 10VM or an external signal can be selected. Amplifier U62 subtracts from I2 a signal proportional to the leakage current through the seal resistance.

Low-noise supplies: The +13V and -6V outputs have very low noise and hum. For the +13V output, regulation is provided by U54A and Q3, Q2. Q4 provides short-circuit and foldback current limiting. The -6V output operates similarly.

Reference potentials: ±5V references (+VREF, -VREF) are used throughout the Axopatch. The positive reference is generated in U23. Amplifier U19A filters and buffers this voltage. Amplifier U19B inverts it to provide the negative reference.

Output calibration: A 100 mV internally generated calibration pulse can be put onto the VM, I and Scaled Outputs. This pulse starts as a 1 V signal on the output of U41A. Timing is provided by switch U44B. A proportional calibration signal can be used by connecting a signal source to the EXT. CAL. SIGNAL connector.

Audio monitor: This circuit is based on the voltage-controlled oscillator U46. U47 is used to scale and offset the control voltage.

Main panel meter: Each of the 4 inputs is scaled so that the display reads mV and pA as appropriate.

Power input: Connector J5 brings power to the main board from the power supply board (Assembly A1001 described later).

RMS Meter (refer to A-12)

The heart of the RMS meter is the AD636 (U51) RMS-to-DC convertor. The input to this integrated circuit passes through a 4-pole lowpass Butterworth filter with -3 dB frequency at 5 kHz. Digital display is generated in the ICL7107 (U53) voltmeter integrated circuit.

Thumbwheel-to-Voltage Convertor (refer to A-17)

The setting of the thumbwheel switch establishes the current output of the multiplying digital-to-analog convertor A1. A2 converts the current output into a voltage output. The polarity and maximum output depends on the polarity and amplitude of the reference voltage connected to V_{in} of A17.

Power Supply (refer to A-13)

Located in the left-hand section of the instrument.

PLEASE NOTE**: Line voltages are present in the power-supply section.**

The line input connector contains an RFI filter, the fuse in use and a spare fuse. A slide switch on the circuit board puts the two primary windings into series or parallel connection for 230V or 115V operation respectively. Line voltages from 200-260V and 100-130V AC are acceptable.

Three regulated outputs are generated: +15V, -15V, +5V. Standard 3-terminal regulators are used. Test points on the circuit board allow the regulator input and output voltages to be conveniently measured. 2 mV of line-frequency noise and 2 mV of wideband noise are acceptable. This noise is rejected by the rest of the circuitry.

Maintenance

Adjustment and repair should only be attempted by skilled electronic technicians or engineers.

Caution

Line voltage is connected to some of the transformer leads and some parts of the power-supply board in the left hand side of the instrument. Always unplug the power cord before attempting to handle or repair these sections.

Layout

Most of the circuitry of the Axopatch is located on a large horizontal main circuit board. On the left hand side there is a vertical daughter board containing circuitry for Track and Command. In the far left, separated by a grounded metal shield, is the power-supply board.

Grounding

Signal ground and chassis ground are isolated from each other.

Access

All test points and trim potentiometers can be accessed by removing the top cover.

All components can be desoldered from the main circuit board without removing the board. Simply remove the bottom cover for access to the non-component side of the board.

Routine Maintenance

Routine maintenance is not required. The adjustment procedure should be performed after repairs to the main circuit board but not otherwise.

Adjustment Procedure

Abbreviations

TP; test point
V_{p-p}; volts peak-to-peak
gnd; ground
Scope; oscilloscope
DVM; digital volt meter
DPM; digital panel meter
Thumbwheel on EXT.; activate STEP COMMAND with external logic level signal.
G; headstage gain
α; output gain

Equipment Required

Scope with 100 µV/div resolution
DVM
Sine and Square-wave generator

Trim Procedure

It is important to do the trims in the order presented because some of them rely on earlier trims. For brevity, only changes to the previous setup are listed for each trim. The procedure assumes only one waveform generator is being used.

Measurement Techniques

Filtering

When measuring zero volts or establishing a null the input to the scope should be lowpass filtered at 10 kHz or less.

Measuring DC Voltages

To eliminate offset errors from the measurement all of these measurements will be balanced. That is, the command potential will be switched alternately from +ve to -ve and the difference in the measured outputs will be measured. Connect DVM ground to yellow ground plug at rear of unit.

References and Commands

ß = 1/0.1
Remove headstage
Using suitable jumper, connect pin 19 to pin 20 on J1 (designated clamp headstage on rear of Axopatch)
Mode = V-CLAMP
Thumbwheel = 100.0 CONT.
Using DVM monitor TP1 (on Track board)
Repetitively switch from + to -
☐ Trim RT4 for ±5.00 V

Using DVM monitor TP4
☐ Trim RT1 (on Track board) for ±2 V

Select I on DPM
Link TP1 (on Track board) to TP9 (on main board)
Repetitively switch from + to -
☐ Trim the pot inside DPM for ±100.0 pA
Remove link

Using DVM monitor TP13
Thumbwheel = 000.0
Use Manual Junction Null for zero reading
Thumbwheel = 100.0 CONT.
Repetitively switch from + to -
☐ Trim RT5 for ± 100 mV

Using scope monitor TP14
Thumbwheel = 000.0 Osc., 100 Hz
AC couple scope
Scope on 100 µV/div, 10 kHz
☐ Trim the pot on back of thumbwheel board for no step

Using DVM monitor TP17
Output Cal = CONT.
☐ Trim RT7 for +1 V
Output Cal = Off

Thumbwheel = 100.0 CONT.
DVM on TP23
Repetitively switch from + to -
☐ Trim RT10 for ±1000 mV
Thumbwheel = 0 Off

RMS Meter

Select EXT. (mV)
☐ Trim RT8 for zero reading
Apply 1 kHz sine wave to Input to RMS Meter input
DVM on TP20
☐ Adjust source magnitude for accurate 100 mV RMS on TP20
☐ Trim RT16 for ten times DVM reading
Remove external signal

Audio Monitor

Volume on
Output Select = Vm
Check Vm = 0 on DPM
Scope on TP18
☐ Trim RT9 for 450 µs period
Volume = OFF

Output Section

Select α = 100
Select Bypass
Scope on TP26
☐ Trim RT17 for zero DC

Use external source to activate Reset Auto Output Zero at 1 kHz
Select Auto Output Zero = Active
α = 1
Scope on TP26, 20 mV/div
Thumbwheel = 100.0 CONT.
Repetitively switch from + to -
☐ Trim RT14 for zero spread
☐ Trim RT15 for zero offset
Auto Output Zero = OFF
Remove external source

Whole-Cell Compensation

Select % Comp.= OFF
Select ß = 1/0.1
Thumbwheel = 010.0 OSC., 10 Hz
Scope on TP2A
Ground junction of R75A, B, C
Series Resistance = 50 MΩ, Whole-Cell Capacitance = 50 pF
Check that signal is about 100 mV
Select % COMPENSATION = 100%
☐ Trim RT3 for fastest rise without overshoot
Select % COMPENSATION = 0%
Remove ground from junction of R75A, B, C
Thumbwheel = 000.0 OFF

R Series Correction

Connect square-wave source to TP9, 1 kHz, 1 V_{p-p}
Scope on TP13
Select ß = 1/0.1, Series Resistance = 100 MΩ
Select 100% COMPENSATION
☐ Trim RT1 for 200 mV_{p-p}
Remove source
Switch % COMPENSATION and Capacitance Compensation controls off

Headstage (This step should only be performed using a CV-4-1/100U headstage)
Scope filter at 10 kHz
Connect CV-4-1/100U headstage to ground via 10 MΩ and 50 pF in series
Thumbwheel on 050.0 OSC., 100 Hz
Scope on TP8
Use Capacitance Compensation controls to obliterate transient
Set % COMPENSATION = 80%
☐ Trim RT2 for minimum slow transient
Set Capacitance Compensation controls and % COMPENSATION = OFF
Thumbwheel = 000.0 OFF

Current Clamp
Select ß = 1/0.1
Remove headstage if connected
V-CLAMP mode
Scope on TP16, 100 µV/div
☐ Trim RT6 for 0V

I-CLAMP mode
Replace headstage
Remove jumper from pins 19 and 20 of J1
Scope on TP23
Connect headstage to an external signal source via 1% resistor for CV-4-1/100U use 10 MΩ, for CV-4-0.1/100U use 1 MΩ.

Set source = 100 mV_{p-p} accurate, 100 Hz
☐ Trim RT12 for 1 V_{p-p}

Link test point near J2 (bath headstage connector) to ground
Connect 1 V_{p-p} accurate 100 Hz source to TP3
☐ Trim RT13 for 500 mV
Remove link

Thumbwheel = 100.0 OSC., 100 Hz
Set Series Resistance to read 0 MΩ
Set Series Resistance to read 10 MΩ
☐ Trim RT11 to null response
Select V-CLAMP mode
Thumbwheel = 000.0 OFF

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Further Reading

Rae, J.L., Levis, R.A. (1984). *Patch voltage clamp of lens epithelial cells: Theory and practice.* Molec. Physiol. 6, 115-162.

Sakmann, B., Neher, E. (1983). **Single-Channel Recording.** New York: Plenum Press.

WARRANTY

We warrant every Axopatch-1D and every headstage to be free from defects in material and workmanship under normal use and service. For 12 months from the date of receipt we will repair or replace without cost to the customer any of these products that are defective and which are returned to our factory properly packaged with transportation charges prepaid. We will pay for the return shipping of the product to the customer.

Before returning products to our factory the customer must contact us to obtain a Return Merchandise Authorization number (RMA) and shipping instructions. Failure to do so will cause long delays and additional expense to customer. Complete a copy of the RMA form on the next page and return it with the product.

This warranty shall not apply to damage resulting from improper use, improper care, improper modification, connection to incompatible equipment, or to products which have been modified or integrated with other equipment in such a way as to increase the time or difficulty of servicing the product.

This warranty is in lieu of all other warranties, expressed or implied.

Axon Instruments, Inc.