Tools for Physiology Labs: An Inexpensive High-Performance Amplifier and Electrode for Extracellular Recording

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Abstract
The cost of electronic equipment can be a critical barrier to including neurophysiology exercises in biology teaching programs. We describe the construction of a simple and inexpensive AC preamplifier with performance comparable to that of commercial products. The amplifier consists of two integrated circuits in five stages: differential input, fixed gain, variable gain (100 or 1000), low-pass filter (5 or 20 kHz), and 50 or 60 Hz notch filter. We compared our amplifier with two commercial units, the A-M Systems Model 1700 and the Grass P15. The quality of extracellular recording from a typical student preparation (spontaneously active crayfish motor nerve) was the same for all three amplifiers, although our amplifier has slightly higher internal noise than the P15 and slightly lower common-mode rejection than the 1700 and P15. In addition, we describe a simple suction electrode for extracellular nerve recording. It is easily constructed from readily available materials and uses a disposable plastic pipette tip, instead of the traditional glass tip, to contact the nerve. This tip is easily replaced if broken or clogged, and can be adapted to different recording conditions by selecting a different tip size or stretching the plastic. Development of this equipment is part of an ongoing project to promote neuroscience education by expanding the neurophysiology options available to laboratory instructors.

1. Introduction
We are developing teaching material for the undergraduate neuroscience laboratory. Our goal is to expand the instructional capabilities of faculty teaching physiology, and in particular, to increase the resources for introducing neuroscience into biology curriculum. We have previously presented laboratory exercises that use invertebrates as model systems to teach general principles of nervous system physiology, instead of more traditional vertebrate preparations (Wyttenbach et al., 1999; www.crawdad.cornell.edu). A limitation of adopting these and other neurophysiology exercises in many teaching environments is the cost of the electronic equipment required to record electrical potentials from nerves and muscles. Most commercially available equipment is designed for research and is priced accordingly. This can be a significant barrier to setting up a neurophysiology teaching lab. To help overcome this barrier, we are designing inexpensive instrumentation that can be built by faculty, support staff, or even students. In this paper, we describe a low-cost extracellular amplifier with performance comparable to that of research units. We also describe a simple extracellular electrode that our students use with this amplifier to record action potentials from motor and sensory nerves. Both pieces of equipment can be used with either invertebrate or vertebrate preparations.

An extracellular amplifier for use in the student laboratory should have the following characteristics: (1) gains of 100 and 1000, sufficient for most nerve and muscle activity; (2) good frequency response from 300 Hz to 5 kHz, matching the bandwidth of nerve and muscle spikes; (3) 60 Hz or 50 Hz notch filter to reduce interference from line voltages; (4) good common-mode rejection, permitting use in electrically noisy environments; (5) low internal noise; (6) high input impedance to permit use with a variety of electrode types; and (7) low power requirements to allow battery operation for extended periods. To be a practical alternative to commercial units, a home-made amplifier should (1) be significantly less expensive; (2) be straightforwardly constructed, with as few components as possible; and (3) require no adjustments for best performance.

There are many published designs for physiological amplifiers (e.g. Hamstra et al., 1984; Metting VanRijn et al., 1994), but most are designed for specific uses and either do not meet the needs listed above or are complicated to build. The circuit we describe uses only two integrated circuits (ICs) and has the entire differential input stage in a single IC, eliminating the need for component matching and calibration. Details of the circuit design and construction are found in the Methods section.

There are many ways to record nerve, neuron, and muscle potentials from the extracellular fluid surrounding excitable tissue (Sykova, 1992). Suction electrodes, for example, are commonly used to record action potentials from exposed nerves because they contact the nerve relatively gently but can form a tight seal between the electrode tip and the preparation, giving excellent signal-to-noise ratios. In fact, modern electrophysiological methods of patch clamp recording are descended from cruder suction electrode recording techniques (Sakmann and Neher, 1995). A variety of suction electrode designs are documented in the literature and some can be bought commercially (Easton, 1960; Florey and Kriebel, 1966; Delcomyn, 1974; Stys, 1992). Most rely on a pulled, broken, and polished piece of glass tubing as an electrode tip. A glass tip has major disadvantages in the
teaching laboratory: it is easily broken, is difficult to quickly make and replace during a laboratory exercise, and is difficult to make in consistent sizes. To avoid these problems, we designed a simple and inexpensive suction electrode that uses commercial plastic pipette tips instead of glass tubing.

2. Methods

2.1 Amplifier design

The amplifier consists of five logical sections: an input stage, two amplification stages, and two filtering stages (Fig. 1). It is a standard operational-amplifier (op-amp) design using two integrated circuits (for explanation of basic op-amp use, see Horowitz and Hill, 1989). Gains of the sections are: (1) differential input with DC gain of 10, (2) fixed amplification with AC gain of 100, (3) gain of 0.63 or 6.3, (4) low-pass filter with gain of 1.58, and (5) notch filter with gain of 1.0, giving a total gain of 100 or 1000 for the entire circuit. Section 4 is a low-pass filter designed for optimal flatness of frequency response below 5 kHz. The cutoff frequency can be varied by changing R8 and R9 in the input of the third stage. To bypass the low-pass filter, thus extending the bandwidth to 20 kHz, set R6-7 to zero and remove C3-4 (or install an SPST switch between the points marked * in Fig. 1). Section 5 is a 60 Hz notch filter to remove any interference not eliminated by the common-mode rejection of the input section. Although best performance is achieved if capacitors C5-8 are carefully matched, adequate 60 Hz attenuation is achieved with unmatched capacitors of 5% tolerance. For a notch filter of 50 Hz instead of 60 Hz, increase C5-8 to 33 nF.

2.2 Amplifier construction

The amplifier can be constructed on a 2×3-inch printed-circuit board (Fig. 2A) or, using the same layout, on a piece of perfboard. For isolation from electrical noise, it should be housed in a metal enclosure (e.g. Bud aluminum 2-piece minibox CU-3006-A). We suggest using binding posts for the inputs, since they accept banana plugs, pin plugs, or bare wire. For tips on such circuit construction techniques as soldering, see Horowitz and Hill (1989) or the Electronics Express web site (www.elexp.com/tips.htm).

The total cost of parts, excluding an enclosure, is approximately $25 US. The following parts are required (quantity and part number in brackets). See the Notes section for sources.

Burr-Brown instrumentation amplifier INA121 [1]
Linear Technologies quad op amp LT1079 [1]
10 nF capacitor [2; C1-2]
330 pF capacitor [2; C3-4]
27 nF 5% capacitor (33 nF for 50 Hz notch filter) [4; C5-8]
100 kΩ resistor [7; R1, R3, R6-8, R10-11]
1 MΩ resistor [1, R2]
69.8 kΩ 1% resistor [1; R4]
634 kΩ 1% resistor [1; R5]
57.6 kΩ 1% resistor [1; R9]
49.9 kΩ 1% resistor [1; R12]
5.62 kΩ 1% resistor [1; R13]
14 pin DIP socket for LT1079 [1]
8 pin DIP socket for INA121 [1]
9V battery [2]
9V battery clip with leads [2]
BNC jack for output [1]
binding post, red, for + input [1]
binding post, black, for– input [1]
binding post, green, for ground input [1]
SPST switches for gain and filter [2]
DPST switch for power [1]
1.5 inch aluminum stand-offs with screws [2]
2×3 inch printed circuit board (Fig. 2A) or perfboard [1]
Metal enclosure at least 4×2.25×2.25 inches [1]
Fig. 2. Amplifier construction. A. Printed-circuit board, showing lines of copper. B. Reverse side of the board, showing layout of components and wiring of switches, connectors, and batteries. Where the two integrated circuits appear, use sockets rather than soldering the ICs directly onto the board. A switch for the low-pass filter is included in this circuit, bridging the points marked * in Fig. 1. If a fixed low-pass of 5 kHz is desired, omit the switch and make no connections to points k and l. C. Layout of switches and jacks on the enclosure, as viewed from the outside. Crosses indicate locations of holes, fractions are the hole diameters in inches. D. Layout and connection of switches and jacks, as viewed from the inside of the enclosure. Letters beside wires match letters shown in B. E. Completed amplifier, with connections made, ICs in their sockets, and batteries in place. All figures are shown actual size.
Construction and takes about 4 hours (less if building several at once). First, solder the resistors, capacitors, and DIP sockets onto the printed-circuit or perfboard as shown in Fig. 2B. Next, solder the 9V battery clip leads to the circuit board (locations e-h in Fig. 2B), taking care that the polarities are correct (the red lead is positive). Cut 13 pieces of wire approximately 4 inches long and strip 1/4 inch of insulation from each end. Use several colors of wire if possible, since this will help keep them straight later. Solder these wires to the ends of circuit board as shown in Fig. 2B (locations a-d and i-q). Next, mount the switches, binding posts, and BNC jack on the enclosure as shown in Fig. 2C; add the two standoffs inside the enclosure. Now solder the wires dangling from the circuit board to the switches and jacks as shown in Fig. 2D, matching the letters in Fig. 2B. Finally, screw the circuit board to the standoffs with the components facing up. Insert the two ICs into their sockets in the orientation shown in Fig. 2B. Place two 9V batteries in the enclosure beside the circuit board, install their clips, and close the enclosure. If the batteries rattle inside the enclosure, surround them with cardboard pieces. Fig. 2E shows the completed amplifier before the enclosure is closed.

2.4 Amplifier use

The amplifier can be used with a variety of electrode types, including suction, pins, hooks, nerve chamber, and electromyography wires. For bipolar (differential) recording, connect the recording and indifferent electrodes, respectively, to the + and − inputs, with the preparation ground connected to the GND input. For monopolar (single-ended) recording, connect the preparation ground to both the − and GND inputs. Connect the output to an oscilloscope and/or computer. For most nerve recordings, a gain of 1000 and low-pass filter of 5 kHz are appropriate. For best results, keep the amplifier in a Faraday cage with the preparation. If this is not practical, keep wires between the electrodes and amplifier short and use shielded cable if possible.

The amplifier draws less than 2 mA from two 9 V batteries. Assuming standard 0.5 A-hour 9 V batteries, the circuit should run for about 250 hours on one pair of batteries. When batteries are weak, the output signal becomes clipped (spikes are flat rather than pointed at their positive and negative peaks). If battery operation is not practical, an AC-to-DC converter supplying positive and negative 6 to 12 V may be used. However, this risks bringing electrical noise into the amplifier unless the converter is of the highest quality. If a converter is used, it should be grounded and placed outside the Faraday cage so that only insulated DC lines enter the shielded recording area.

2.5 Electrode construction

The suction electrode consists primarily of a replaceable gel-loading pipette tip (a very fine disposable pipette tip, which contacts the nerve), a 1 cc syringe (which fits in a standard manipulator), and a 10 cc syringe (which provides the suction). The positive and negative poles of the electrode are stainless steel or platinum wires. Lengths of tubing and cable are not critical; they can be as long as needed. The following materials are required (see the Notes section for sources):

- 1 cc plastic disposable syringe, plunger discarded
- 10 cc plastic disposable syringe
- 18-gauge syringe needle, tip filed blunt
- Microbore tubing to fit 18-gauge needle
- Stainless steel wire, uninsulated, approximately 0.25 mm diameter
- Two-conductor cable, shielded if possible, of any desired length
- 200 ml pipette tip
- Ultra micro gel-loading pipette tip (0.2 mm diameter)
- Fast-setting glue (hot glue gun works best)

Steps in construction of the electrode are shown in Fig. 3. Brief instructions are given here; a full set of step-by-step instructions can be found in Wyttenbach et al. (1999).

1. Make a hole all the way through the 1 cc syringe near its tip.
2. Remove 10 cm of insulation from a length of cable, exposing the two internal wires.
3. Insert the two wires of the cable into the rear of the syringe so that one wire protrudes through each of the two holes made in step 1; strip about 5 mm of insulation from each of the two wires.
4. Place the 200 ml pipette tip on the syringe.
5. Apply the fast-setting glue to the truncated end of each wire. Insert the truncated end of each wire into the syringe, making sure that no air is trapped inside the syringe. Squeeze the glue around the wires to form a seal.
6. After 1 hour of setting time, cut the wire leaving the syringe so that about 1 cm of the wire protrudes from the syringe.
7. Apply the fast-setting glue to the truncated end of the pipette tip.
8. Insert the truncated end of the pipette tip into the truncated end of the syringe.
9. Place the syringe over the pipette tip and squeeze the glue around the circumference of the syringe to form a seal.
10. Allow the glue to set for 1 hour.

Fig. 3. Suction electrode construction. See the text for a description of each step. A. After step 5, showing wires protruding through holes in the 1 cc syringe. B. Step 6, placing the 200 ml pipette tip on the syringe. C. After step 8, showing the 200 ml pipette tip attached with the internal wire protruding from its tip. D. Final product with disposable gel-loading pipette tip in place and wire coiled around it.
Cut a length of tubing and thread it through the tip of the 1 cc syringe so that it comes out the back end. Pull the tubing most of the way through, leaving about 5 mm protruding from the syringe tip. (5) Solder a 6 cm piece of steel wire to the positive lead of the cable. (6) Thread the 200 ml pipette tip over the steel wire. (7) Glue the pipette tip onto the syringe; the wire should protrude slightly from the end of the pipette tip. (8) Solder the wire (still on its spool) to the negative lead of the cable. (9) Firmly place a gel-loading pipette tip on the 200 ml pipette tip, loosely coil the steel wire around the two pipette tips, and cut the wire. (10) Put the free end of the tubing on a blunt 18-gauge syringe needle and put the needle on the 10 cc syringe.

2.6 Electrode use
Clamp the 1 cc syringe in a micromanipulator. Connect the electrode cable to an amplifier with the lead from the inner electrode wire connected to the positive input and the lead from the outer electrode wire connected to the negative input. With the biological preparation mounted in its recording chamber, lower the electrode tip into the saline bath surrounding the preparation and suck saline up to the level of the internal wire of the electrode (Fig. 3). The outer wire must also rest in the saline bath to complete the circuit. Place the electrode tip onto a nerve and apply suction. See Fig. 6 for extracellular recordings made with this type of electrode.

After use, expel all saline from the electrode; rinse by sucking and expelling water before long-term storage. The electrodes will last longer if stored more-or-less straight, since the main source of failure is a kink or break in the tubing. The gel-loading pipette tip can bend or become clogged, but is easily replaced with another.

2.7 Performance testing
We compared our amplifier to two commercial units that we have used in our teaching laboratory, the A-M Systems Model 1700 and the Grass P15. We used three criteria as measures of amplifier performance: internal noise, common-mode rejection ratio (CMRR), and the quality of an extracellular recording made under standard student recording conditions.

The internal noise of each amplifier was determined by measuring its output with resistances of 0 Ω, 100 kΩ, and 1 MΩ placed across the differential inputs. Ideally, the output voltage should be zero. To measure the CMRR, we applied a 1 V 60 Hz sine wave to both amplifier inputs through 100 kΩ resistors and recorded the output. In this test only, the 60 Hz notch filter was not used. In all tests, and in the recording that follows, all amplifiers were tested at a gain of 1000. The A-M Systems amplifier and our amplifier were set to a bandwidth of 300 Hz to 5 kHz; the Grass P15 was set to 300 kHz to 10 kHz.

We compared the quality of each amplifier output in a typical student setting by recording spontaneous motor action potentials from a crayfish motor nerve. The third nerve of crayfish abdominal ganglia is easily recorded from in the teaching laboratory. Background for this preparation and details of the dissection can be found in Lab 2 of Wytenbach et al. (1999). Briefly, a crayfish was anesthetized in ice and its tail removed. The tail was pinned in a sylgard-lined dish and covered with cold crayfish saline (in mM: 5.4 KCl; 207.3 NaCl; 13.5 CaCl₂; and 2.6 MgCl₂). An incision was made along the midline of the third tail segment and along the left and right edges of the anterior sternite. This exposed the ventral nerve cord, the ganglia under the anterior and posterior sternites of the third segment, and the 3 nerves leaving the anterior ganglion. Nerve 3 contains only the 6 motor neuron axons that innervate the superficial flexor muscle. This is a postural muscle that receives continuous motor activity, even in isolated tail preparations. Differential recordings were made from nerve 3 as described in sections 2.4 and 2.6 above, with a silver chloride pellet in the saline bath as the reference ground. See Fig. 4 for details of shielding and grounding this preparation and equipment; see Ohlemeyer and Meyer (1992) for an introduction to general grounding procedures. Amplifier output was split between an oscilloscope, audio monitor, and computer.

Recordings from the three amplifiers were made with the same suction electrode from the same preparation. The output leads of the electrode were simply moved from one amplifier to another. The commercial amplifiers had not been calibrated since their purchase, as is typical of amplifiers in teaching (and most research) laboratories.

3. Results
The internal noise of our circuit was comparable to that of the A-M Systems Model 1700 at all connecting resistances, slightly higher than that of the Grass P15 at 0 and 100 kΩ, and twice that of the Grass P15 at 1 MΩ (Fig. 5).
The internal noise of all three amplifiers is negligible relative to the physiological signals they are intended to record. The common-mode rejection ratio (CMRR) of our circuit was 75 dB. A CMRR of 20 dB means that the subtraction eliminates 90% of the noise voltage that is applied to both inputs. A common-mode rejection of 40 dB means 99% is removed, and 60 dB means 99.9% is removed. The CMRR of our circuit was not quite as good as that of the other two amplifiers (Fig. 5), but still results in removal of over 99.9% of noise common to both inputs.

Fig. 5 shows extracellular recordings of spontaneous motor nerve activity from the crayfish third abdominal nerve, using each of the three amplifiers. The traces show action potentials of large and small amplitudes, which reflect the wide range of axon diameters of the identified motor neurons in nerve 3 (Kennedy and Takeda, 1965). These recordings were made under identical conditions by merely moving the output leads of the suction electrode from one amplifier to another. There is no obvious difference in the quality of the three recordings, thus the higher internal noise and lower CMRR of our amplifier do not compromise the recording quality relative to the commercial amplifiers. In addition, the traces demonstrate the quality of recording typical of our suction electrode design.

4. Discussion
We present an amplifier design that meets the requirements for high quality extracellular recordings of nerve and muscle in the teaching laboratory. In particular, it has the gain and frequency response needed to reproduce action potentials; has adequate common-mode rejection, notch filtering, and internal noise; has high input impedance; and has low enough power consumption that a pair of batteries should last for an entire semester. In addition, the cost of parts is very low and construction is straightforward, using only two ICs. Because our design uses an integrated instrumentation amplifier at the input, it requires no component-matching or adjustments once the circuit is built. The amplifier is not limited to use with the suction electrode described here. Because of the high input impedance of the amplifier, impedance matching for different electrode types is not required. It is suitable for use with suction, pin, hook, needle, nerve chamber, electromyography wire, and surface electrodes.

For the teaching laboratory, this circuit is an improvement over other published designs. For example, the design of Hamstra et al. (1984) is specialized for low power consumption but has a bandwidth of only 200 Hz, making it unsuitable for neurophysiology. The design of Metting VanRijn et al. (1994) uses more components than ours and requires that resistor and capacitor values be carefully matched to achieve good common-mode rejection.

The amplifier can be easily modified to meet specialized needs. As described, the bandwidth is 160 Hz to 5 kHz. To raise the high-pass cutoff without altering the gain, change C1 and C2 (5 nF for 320 Hz, 1 nF for 1600 Hz) in sections 2 and 3 (Fig. 1). To change the low-pass cutoff without altering the gain, change C3 and C4 (1500 pF for 1 kHz, 150 pF for 11 kHz) in section 4. Closing a switch between the points marked * in Fig. 1 removes low-pass filtering, extending the bandwidth to 20 kHz. This would be useful when recording very fast waveforms such as the discharge of an electric fish (see Lab 1 of Wytenbach et al., 1999). Gain is currently selectable between 100 and 1000. If higher gains are needed (e.g. 1000 and 10000), it is best to achieve this by replacing section 4, the low-pass filter, with a simple amplification stage with a gain of 10, duplicating section 2. In that case, R5 should be changed to 1 MΩ and R4 to 110 kΩ, giving a gain of 1 or 10 in section 3. If lower gains are needed (e.g. 10 and 100), change R2 to 100 kΩ. Finally, to set the notch filter to 50 Hz instead of 60 kHz, increase C5-8 to 33 nF.

We routinely use the suction electrode described here in our teaching laboratory, and Fig. 6 shows the quality of recordings possible with this design. The greatest advantage

<table>
<thead>
<tr>
<th>Amplifier</th>
<th>Internal Noise (µV RMS)</th>
<th>CMRR at 60 Hz</th>
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<tbody>
<tr>
<td>Grass P15</td>
<td>1.4</td>
<td>&gt;80 dB</td>
</tr>
<tr>
<td>A-M 1700</td>
<td>2.5</td>
<td>&gt;100 dB</td>
</tr>
<tr>
<td>Land et al.</td>
<td>2.1</td>
<td>75 dB</td>
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<td></td>
<td>0 Ω, 100 kΩ, 1 MΩ</td>
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Fig. 5. Noise and common-mode rejection comparisons. Internal noise is the output of the amplifier (root-mean-square, RMS) with a resistance of 0 Ω, 100 kΩ, or 1 MΩ placed across its inputs. Ideally, this should be zero. The values for all three amplifiers are negligible relative to the physiological signals they are intended to record. Common-mode rejection ratio (CMRR) is the ratio of output to input with a 1 V, 60 Hz sine wave applied to both inputs. A CMRR of 20 dB means that 90% of the common signal is eliminated, 40 dB means 99% is eliminated, and 60 dB means 99.9% of the common signal is eliminated.

Fig. 6. Extracellular recording comparison. Recordings of spontaneous activity in nerve 3 of a crayfish abdominal ganglion were made with each of three amplifiers. The same electrode placement was used in each recording; the electrode cable was simply switched between amplifiers. All traces are shown at the same scale (see scale bar); each amplifier is named to the left of its trace, where Land et al. refers to the circuit described in the current paper.
of using these electrodes over glass-tipped ones is the easy replacement of the recording tip that contacts the nerve. We use commercial ultra micro pipette tips that are easily replaced when they bend or become clogged. Tips of different pore diameters can be purchased to match the recording tip to the nerve diameter for the best signal-to-noise ratio. In addition, the diameter of the ultra micro pipette tip can be easily adjusted by warming the tip between one’s fingers, stretching it and then cutting it back for the appropriate tip-nerve interface (new plastic tips stretch better than older ones). Applying petroleum jelly to the electrode tip after contact with the nerve will also increase the signal to noise ratio. Devoting part of a laboratory session to having students make their own recording electrodes may help focus discussion on the sequence of charge transfers that occur from the time an action potential is generated in a nerve to its appearance on the oscilloscope or computer (Loeb & Gans, 1986). Variations on suction electrode designs that are used in research may allow even higher resolution recordings for student research projects (Wilkins & Wolfe, 1974; Heinzel et al., 1993). A student electrode that is somewhat similar in design to ours, in that it uses a disposable plastic syringe as the electrode shell and can use replaceable tubing that matches nerve diameters, is described by Easton (1993). However, Easton’s electrodes are part of specialized preparation chambers designed to stimulate and record action potentials from frog nerves and isolated muscles, and thus may not be as versatile as ours.

Faculty, support staff, and students, as part of a course or for special projects, could construct our amplifier and suction electrode with readily available components. We hope that the availability of homemade equipment design will expand the instructional capabilities of physiology teachers. Low-cost, high-performance equipment design may also be of interest to researchers with limited budgets.

Notes
Most amplifier components are readily available from a variety of suppliers, including Radio Shack (www.radioshack.com), Allied Electronics (www.alliedelec.com), and DigiKey (www.digikey.com). Precision resistors and the Burr-Brown INA121 are stocked by DigiKey. Readymade amplifiers based on our design can be purchased from Edvotek, Inc. (www.edvotek.com).

Parts for the suction electrode are available from most scientific supply dealers, since none of the specifications are critical. Ultra micro gel-loading pipette tips and 200 µl pipette tips are available from Laboratory Product Sales (www.lpsinc.com, parts LL112402 and LL142112, respectively). Steel and platinum wire are available from A-M Systems (www.a-msystems.com). Microbore tubing to fit an 18-gauge needle can be found at Cole-Parmer (www.coleparmer.com, part U-06492-5).

See www.crawdad.cornell.edu for downloadable pdf and eps files of the printed-circuit board, a more complete list of sources with addresses and telephone numbers, and a parts list with catalog numbers and approximate prices. See Wytenbach et al. (1999) for a variety of student laboratory exercises that use this equipment.

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