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Breathable, large-area epidermal electronic systems for recording electromyographic activity during operant conditioning of H-reflex

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Abstract

Operant conditioning of Hoffmann's reflex (H-reflex) is a non-invasive and targeted therapeutic intervention for patients with movement disorders following spinal cord injury. The reflex-conditioning protocol uses electromyography (EMG) to measure reflexes from specific muscles

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Credit Author Statement

Y.-T.K., J.J.S.N., and W.-H.Y. conceived and designed the research; Y.-T.K., J.J.S.N., T.F., H.-R.L., S.K., J.J.C., H.S.K., and J.W. performed the experiments; Y.-T.K., J.J.S.N., H.S.K., Y.C.J., and W.-H.Y. analyzed the data; Y.-T.K., A.C., J.R.W., J.J.S.N., and W.-H.Y. wrote the paper.

¹These authors contributed equally to this work.

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Declaration of interests

Georgia Tech has a pending US patent application.

Appendix A. Supplementary Information

Supplementary data associated with this article can be found in the online version.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

elicited using transcutaneous electrical stimulation. Despite recent advances in wearable electronics, existing EMG systems that measure muscle activity for operant conditioning of spinal reflexes still use rigid metal electrodes with conductive gels and aggressive adhesives, while requiring precise positioning to ensure reliability of data across experimental sessions. Here, we present the first large-area epidermal electronic system (L-EES) and demonstrate its use in every step of the reflex-conditioning protocol. The L-EES is a stretchable and breathable composite of nanomembrane electrodes (16 electrodes in a four by four array), elastomer, and fabric. The nanomembrane electrode array enables EMG recording from a large surface area on the skin and the breathable elastomer with fabric is biocompatible and comfortable for patients. We show that L-EES can record direct muscle responses (M-waves) and H-reflexes, both of which are comparable to those recorded using conventional EMG recording systems. In addition, L-EES may improve the reflex-conditioning protocol; it has potential to automatically optimize EMG electrode positioning, which may reduce setup time and error across experimental sessions.

Keywords

Large-area epidermal electronic system; Electromyography; Multi-channel nanomembrane electrodes; Neuromuscular response; Breathable fabric

1. Introduction

Operant conditioning of the H-reflex is a non-invasive and targeted therapy for improving movement after spinal cord injury (SCI) and other neuromuscular disorders. In this therapy, transcutaneous electrical stimulation elicits a reflex response from a target muscle (Aloraini et al. 2015; Kuck et al. 2018; Li et al. 2017b; Thompson and Wolpaw 2019). The size of this reflex response is quantified, and patients can learn to change the size of their reflex over time when they are rewarded for doing so (i.e., through operant conditioning). In people in whom a partial SCI causes exaggerated reflexes and impaired locomotion, learning to reduce the size of their soleus H-reflex induces beneficial plasticity, increases walking speed and step symmetry, and reduces limping (Thompson et al. 2013). The H-reflex is measured by EMG, which is recorded with metal electrodes kept in contact with the skin by conductive gel-embedded adhesive pads (Budini et al. 2018; Lou et al. 2017; Özyurt et al. 2018). During use, the gels on these rigid electrode pads gradually dry out; this increases electrical impedance and reduces signal quality (Lee et al. 2014; Li et al. 2017a; Mishra et al. 2017). In addition, the poor biocompatibility and low breathability of the acrylate-based tape used to secure EMG electrodes to the skin can cause skin itchiness, dryness, and redness (Kim and Rogers 2008; Miyamoto et al. 2017; Yeo et al. 2013). This is especially relevant for those likely to benefit from H-reflex operant conditioning, such as older patients (e.g., individuals who have a stroke) who are at an increased risk of skin breakdown (Duncan et al. 2005; McGough-Csarny and Kopac 1998; Winstein et al. 2016). There are recent studies that demonstrate advances in wearable systems for recording of electrophysiological signals with dry, skin-like electrodes (Byun et al. 2019; Choi et al. 2018; Kim et al. 2019; Kwon et al. 2018; Mahmood et al. 2019). However, none of these devices enables to record the EMG over a large surface area with multiple gel-free electrodes as well as to monitor the operant conditioning of the H-reflex, which is generated from the skin electrical stimulation. The

most recent article in epidermal electronics demonstrates a large-area MRI-compatible sensor interface for recording of EMG and electroencephalograms (Tian et al. 2019). However, this work shows no study on electrical stimulation, H-reflex elicitation, or H-reflex operant conditioning.

Here, we introduce the large-area epidermal electronic system (L-EES); and we demonstrate its use for operant conditioning of the H-reflex. The L-EES is a composite with three layers—a nanomembrane layer containing sixteen 7 mm by 9 mm EMG electrodes in a 4×4 array, an elastomer layer, and a strain-limiting fabric layer. The device is soft, breathable, and stretchable; thus, it is ideally suited for direct mounting on the human skin. It is also durable and can be cleaned and re-used multiple times without losing the quality of recorded signals. We verify through infrared thermography and cell cytotoxicity testing that the L-EES is biocompatible and we show that it can record high-quality multi-channel EMG activity over a large surface area. We then establish the clinical utility of the L-EES by using it to record EMG responses to transcutaneous stimulation (i.e., direct muscle response (M-waves) and H-reflexes) during each component of the spinal reflex-conditioning protocol. Finally, we present evidence that the L-EES improves the spinal reflex operant conditioning protocol. All the participants reported that the device is very comfortable. Furthermore, the multielectrode design eliminates the need for precise EMG electrode positioning because, in each session, the best recording electrode can be automatically selected from the array.

2. Materials and Methods

2.1. Fabrication of the L-EES

The L-EES is a composite device for recording EMG. Several microfabrication methods (i.e., photolithography, wet/dry etching, metal deposition, and material transfer printing) were used to fabricate the three layers of the composite. Details of the processes for fabricating the nanomembrane electrodes and integrating them into the elastomer are described in our prior works (Fan et al. 2014; Mishra et al. 2017). The nanomembrane electrode layer has three sub-layers (Fig. 1A): a polyimide (PI; 1 μm -thick) encapsulation sub-layer; the gold (Au; 200 nm-thick) electrodes; and a second PI (2.5 μm -thick) support sub-layer. The sub-layers are deposited and patterned via lithography on a 100-nm-thick sacrificial layer of polydimethylsiloxane (PDMS) on a Si wafer. Before transfer printing of the patterned sensors, we perform electron beam evaporation of Cr/SiO₂ and UV-ozone treatment to provide covalent bonding and strong adhesion between the nanomembrane electrode layer and the soft composite substrate of elastomer (100- μm thick) and fabric (1-mm thick). The fabric is composed of 48% polyamide, 36% polyester, and 16% lycra. The electrodes are then connected to a flexible thin-film connector (3M 3431-1002 Connector). Additional fabrication details are in Supplementary Note S1, Fig. S1, and Fig. S2.

2.2. Computational mechanics modeling

We conducted three-dimensional finite element analysis (FEA) modeling using ABAQUS (Dassault Systemes Simulia Corporation), which was used to study the mechanical behavior of the stretchable nanomembrane electrode on elastomer/fabric membranes. We designed the L-EES with open-mesh geometries and a multi-layered structure to permit mechanical

flexibility and stretchability, which enabled multiple uses without losing the quality of EMG signals and mechanical fracture. To compute the boundary conditions, we assigned the following material properties: Young's modulus (E) and Poisson's ratio (ν) - $E_{Au} = 78$ GPa and $\nu_{Au} = 0.44$; $E_{PI} = 2.5$ GPa and $\nu_{PI} = 0.34$; $E_{silicone} = 69$ kPa and $\nu_{silicone} = 0.49$; $E_{fabric} = 391$ kPa and $\nu_{fabric} = 0.40$ (Fan et al. 2014; Hong et al. 2013; Jang et al. 2014; Lee et al. 2018).

2.3. Study of biocompatibility via cell viability tests

We studied the cytotoxicity of the L-EES on human cells by culturing human primary keratinocytes on three different substrates (polystyrene petri dish (control); the composite of elastomer and fabric without the nanomembrane electrode layer; and L-EES) in a standard tissue culture incubator at 37 °C with 5% CO₂. In the incubator, the material samples were placed in a 24-well plate and 5000 keratinocytes/cm² were seeded on top of the samples. After 7 days in the incubator, the keratinocytes were washed briefly with the phosphate-buffered saline (PBS), and 0.9 ml of the cultured medium was mixed with 0.1 ml of PrestoBlue (cell-viability detection reagent). The keratinocytes and the reagent were stored in the 37 °C incubator for an additional 10 min. The supernatant was then aliquoted in a 96-well plate for further analysis. A multi-mode microplate reader (SpectraMax, M3) with an absorbance value of 570 nm was used to quantify cell viability under the three different conditions.

2.4. Recording of physiological signals with human participants

We completed two types of experiments with human subjects. The first experiment was conducted at Georgia Tech (IRB# H17212; three participants ages 18 to 40) and the second experiment was conducted at the National Center for Adaptive Neurotechnologies (IRB# 05-058; three participants ages 22 to 36). Prior to the experiments, participants provided written informed consent. In Experiment #1, we compared EMG and motion artifacts recorded from traditional gel electrodes versus the L-EES. We used the L-EES to create forearm EMG activity maps for several different gestures and demonstrated the reusability of the L-EES over 10 times. In Experiment #2, we studied the use of the L-EES to record EMG during each component of the H-reflex operant conditioning protocol applied to the forearm muscle flexor carpi radialis (FCR) of three participants. Experiment #2 comprised two sessions. In the first session, the L-EES was used to record EMG; in the second, traditional EMG electrodes were. The participants sat in a custom experimental chair with an attached table that supported their right forearm on a horizontal pad. The table was adjusted so that the participant's shoulder and elbow were flexed to ~80 and ~15 degrees, respectively, while the wrist was in the neutral position. The L-EES (Session 1) or traditional EMG electrodes (Session 2) were placed over the FCR. A ground electrode was placed on the radial styloid process and a reference electrode over the FCR tendon (~halfway between the ground electrode and the belly of the FCR). EMG activity was recorded with a 16-channel g.USB (g.tec Medical Engineering GmbH, Austria) B-series amplifier and digitized at 1200 Hz. For participant HR-02, a Bortec EMG amplifier (3200 Hz; 10—1000 Hz pass band) was used to record EMG from the traditional electrodes. H-reflexes were elicited through transcutaneous electrical stimulation with a DigiTimer DS8-R (DigiTimer Ltd, Hertfordshire, United Kingdom) constant current stimulator. Online data collection was

controlled by the Evoked Potential Operant Conditioning System (EPOCS), which is built on BCI2000 (Schalk et al. 2004). In Session 1, we first recorded the maximum voluntary contraction (MVC) and an M-wave/H-reflex recruitment curve (RC). Then, the L-EES was moved ~1.5 cm laterally (the inter-electrode distance) and another MVC and RC were recorded, followed by two blocks of 50 H-reflex elicitation trials (HrTs). In Session 2, an MVC, RC, and two blocks of HrTs were recorded. The H-reflex conditioning protocol is fully described in the previous study (Thompson et al. 2009); additional details of H-reflex measurement are in Supplementary Note S2.

3. Results and Discussion

3.1. Design, architecture, and applications of L-EES system

Fig. 1 captures the overview of the L-EES in materials, structures, and application targets. Fig. 1A depicts the multi-layered, stretchable L-EES: the nanomembrane electrodes with polyimide (PI) sub-layers above and below the sub-layer of 7 mm x 9 mm gold electrodes; the silicone elastomeric membrane; and the strain-limiting fabric. For each electrode, the network of repeating circular islands with open-mesh serpentines provides mechanical flexibility and stretchability for conformal mounting on the skin (Fig. 1B). Furthermore, since the deformation of the fabric requires significant force, the mesh fabric limits the strain on the substrate. The result is that the L-EES maintains mechanical compliance and non-abrasive adhesion, even at high levels of strain (Jang et al. 2014). Another key L-EES design feature is the breathable system that integrates a soft, adhesive elastomer and an array of electrodes for conformal lamination onto the skin. Details of the fabrication of the L-EES appear in Materials and Methods, Supplementary Note S1, Fig. S1, and Fig. S2. An illustration in Fig. 1C shows how the L-EES, reference electrode, ground electrode, and stimulating electrodes are mounted on the arm during operant conditioning of the FCR H-reflex. The median nerve stimulation electrodes are placed near the center of the cubital fossa and on the medial surface of the upper arm. Figs. 1D and E give an overview of the H-reflex. Low amplitude median-nerve stimulation excites Ia afferent fibers and thereby elicits the FCR H-reflex (Dixit et al. 2015; Hoque et al. 2018; Pierrot-Deseilligny and Burke 2005). If the stimulation is a little stronger, it also directly activates the largest α -motoneuron axons and thereby elicits a direct muscle response, or M-wave. Flow chart in Fig. 1F illustrates the pathway of the signals involved in the recording of the M-wave (*blue* boxes) and H-reflex (*red* boxes).

3.2. Computational and experimental study of mechanical behavior and breathability of L-EES

The computational analyses and experiments summarized in Fig. 2 show the mechanical behavior and breathability of the L-EES. The experimental results in Fig. 2A compare the mechanical behaviors of three different substrates: a fabric-only substrate; an elastomer/fabric substrate; and an elastomer/fabric/electrode substrate (i.e., the complete L-EES). All these materials show linear elastic behavior with a similar mechanical modulus up to 70% strain, indicating that the mechanics of the fabric dominates the entire mechanics of the multi-layered system. The calculated values of effective mechanical modulus for the fabric, the composite, and the electrode-on-composite substrates in Fig. 2A are 188.9 ± 5.8 , $183.4 \pm$

4.9, and 184.5 ± 6.1 kPa, respectively; thus, they approximate the stiffness of epidermis (Jeong et al. 2013; Yeo et al. 2013). Since the fabric begins to fail at deformations beyond 70%-strain (Supplementary Fig. S3), the mechanics of L-EES is maintained even in extreme situations, such as during skin deformation or lamination/delamination of the L-EES onto the skin. Given the elastic deformation limit (20-30%) of human skin, the fabric-based composite has sufficient mechanical stability (Supplementary Fig. S3) (Hong et al. 2013). We used three-dimensional FEA to design the L-EES to remain mechanically stable following mechanical deformation from handling, skin lamination, and repeated use (Fig. 2B). We then experimentally verified the compliant structure of nanomembrane electrode at the strain-limited point (Fig. 2C). The computational modeling showed the stretchability over 70% of uniaxial tensile strain with the calculated maximum principal strain of less than the fracture limit, 1% (Fig. 2B). Optical microscopic observations (Fig. 2C) validated the structural integrity of the L-EES when a tensile load was applied; there were no mechanical fractures in the structure. Quantitative electrical measurements during repeated stretching of the device (100 cycles; Fig. 2D) support the results of the visual inspection in Fig. 2C. The relative resistance value increased when tensile loading was applied (magnified view of the first 10 cycles; Fig. 2E), but the linear fit (Fig. 2F) had a coefficient of determination of $R^2 = 0.99$. In sum, computational and mechanical tests show that the L-EES is a mechanically stable device—it does not fracture or delaminate—even after undergoing excessive deformations, including stretching, twisting, and rolling (Figs. 2G–I). Given its large areal coverage of the skin, the L-EES's breathability is very important; it allows for long-term, continuous EMG recording without skin breakdown (Herbert et al. 2018; Lee et al. 2018; Mishra et al. 2017). Materials with high water vapor transmission (WVT) allow the skin to breath and exchange air, thereby reducing skin irritation, rash, and redness, and increasing comfort (Miyamoto et al. 2017; Sun et al. 2018). Quantitative measurements of WVT (Supplementary Fig. S4) demonstrated the gas permeability of the L-EES. We compared the evaporation of water from bottles in four conditions: (1) covered by an electrode/elastomer/fabric membrane (i.e., the complete L-EES); (2) covered by an elastomer/fabric membrane; (3) covered by Tegaderm (a clinical-grade medical film (3M) that is widely used at hospitals because its excellent breathability enables long-term use (Kim et al. 2019)); and (4) uncovered (Supplementary Fig. S5). The four bottles were placed in a temperature- and humidity-controlled environment (25 °C and 35%, respectively) for 5 days (Supplementary Fig S4A). WVT values were then calculated (Supplementary Figs. S4B, C) with the following equation (Hu et al. 2019):

$$WVT = \frac{G}{t \cdot A} = \frac{(G/t)}{A} \quad (1)$$

where G , t , and A are the weight change, elapsed time, and test area, respectively. As expected, water evaporation from the covered bottles was less than from the open bottle. Most importantly, the WVT value of the L-EES (3.13 ± 0.18 g/h·m²) was comparable to that of the Tegaderm (3.43 ± 0.18 g/h·m²). The similar gas permeabilities of the L-EES to the Tegaderm suggest that the L-EES should enable long-term EMG recording without causing skin breakdown.

3.3. Biocompatibility study of L-EES via cytotoxicity evaluation

Fig. 3 summarizes the skin-biocompatibility testing with the L-EES. First, we evaluated the cytotoxicity of our materials in cultured human epidermal keratinocyte cells. After a 7-day exposure in culture to substrates (either electrode/elastomer/fabric or elastomer/fabric) or to no substrates, the cells were identified in a viability assay as dead (having taken up blue dye) or alive (excluding blue dye) by fluorescence microscopy (Supplementary Figs. S6A–C). Based on cell counting, all the samples show viability comparable to the control culture (over 80% live cells in all cases; Supplementary Fig. S6D). In addition, Supplementary Fig. S6E shows that cell proliferation rates were similar for the control and L-EES (SpectraMax M3 microplate reader for an optical absorbance reading at 570 nm). The summary table in Supplementary Fig. S6F clearly demonstrates the biocompatibility of the L-EES; it does not have cytotoxic effects on these cultured human cells. In addition, *in vivo* skin assessment by visual observation and infrared thermography of the L-EES contact sites did not find adverse effects of the device on the skin (Figs. 3A and 3B). Both the L-EES and a traditional gel electrode were mounted on the forearm for EMG recording. After eight hours, only the gel electrode produced erythema (i.e., skin redness and elevated temperature). Fig. 3C reveals the soft, breathable arrangement of L-EES upon skin lamination, showing a possible long-term study of EMG and corresponding neuromuscular response. Integrating the nanomembrane electrodes with elastomer and fabric makes the device breathable, enabling short- and long-term wearability.

3.4. L-EES for a 16-channel EMG mapping and minimized motion artifacts

Fig. 4 summarizes experiments with human participants that compared EMG recorded by traditional electrodes with those recorded by each of the 16 electrodes in the L-EES. The distance between the centers of the L-EES electrodes was 11 mm transversely and 16 mm longitudinally (Fig. 4A). The conformal lamination of the L-EES was demonstrated by recording forearm EMG while participants made five gestures: rest (open hand); closed hand; thumb movement; middle and ring finger flexion; and wrist flexion/extension (details of gestures in Supplementary Fig. S7). For all five gestures, all 16 L-EES electrodes recorded good EMG data (Fig. 4B). We also compared the signal-to-noise ratio (SNR) of EMG recorded by the L-EES to that of EMG recorded by an array of 16 traditional electrodes placed in the same locations as each of the L-EES's 16 electrodes (Fig. 4C). SNR was calculated as (Kwon et al. 2018; Kwon et al. 2019):

$$SNR = 10\log_{10}\left[\left(\frac{A_{signal}}{A_{noise}}\right)^2\right] \quad (2)$$

where A_{signal} was the average full-wave rectified EMG recorded during each gesture, and A_{noise} was the average full-wave rectified EMG during rest. As summarized in Supplementary Fig. S8, the L-EES had comparable SNR to the traditional electrodes, without being encumbered by a tangle of 16 wires and the use of gels. To validate the reusability of the L-EES, we placed the L-EES on a participant, recorded EMG from it, and removed it 10 times (Fig. 4D). Because we marked the initial position of L-EES on the arm, consistent positioning is possible during the reuse test. The SNR of L-EES EMG is similar across the 10 iterations, indicating that the device maintained its structural integrity across

multiple mountings/removals. In contrast, the SNR of the EMG recorded by the traditional electrodes fell after only one iteration (Supplementary Fig. S9). Unlike the acrylate-based tape used by the traditional electrodes, the soft elastomeric substrate on a breathable fabric (i.e., the L-EES) provided a skin-friendly, hydrophobic contact that minimized exfoliation of the stratum corneum. Thus, the L-EES maintained adhesion and skin-electrode contact quality. In addition, to assess the effect of motion artifacts on the L-EES versus traditional electrodes, we analyzed EMG recorded from the forearm during wrist flexion while a participant was motionless or while they were walking (Supplementary Fig. S10). Natural walking that includes arm swing reduces the SNR for both the L-EES and the traditional electrodes; however, the reduction was minimal for the L-EES. This is a major advantage of the L-EES, since motion artifacts are a major problem in EMG recording (Herbert et al. 2018; Herbert et al. 2019). This difference probably reflects the instability of the traditional electrode's gel/skin interface and/or torsion cause by the 16 (often-tangled) wires. Overall, the results demonstrate the excellent EMG-recording performance of the L-EES. Future work could explore optimization of the parameters of the L-EES, including reducing electrode size and interelectrode distance (in accordance with the recommendations of Loeb and Gan 1986)(Loeb et al. 1986) as well as overall patch size.

3.5. EMG monitoring during the H-reflex operant conditioning protocol

Fig. 5 shows the experiments using the L-EES to record the H-reflex for spinal reflex operant conditioning. The experimental setup is shown in Fig. 5A; the L-EES is the black patch of fabric on the participant's forearm (i.e., over the FCR). The ground electrode (green) is on the radial styloid process and the reference electrode (white) is on the FCR tendon. The stimulation electrodes are located proximally to the L-EES near the cubital fossa (green and red electrodes in Supplementary Fig. S11). The tabletop, arm pad, and structure to maintain stable posture surround the participants arm; the EMG amplifier is in the background. Fig. 5B is a screenshot of the operator interface screen (Evoked Potential Operant Conditioning System (EPOCS)); additional details are in Supplementary Fig. S12). On the left side, a bar graph (which is also shown on the participant's interface screen) indicates the level of FCR EMG activity. This bar is updated every 100 ms. By keeping the green bar in the shaded area, the participant maintains a constant level of EMG activity throughout the study; this EMG maintenance is required to elicit stimulation. The waveforms from the most recent stimulation are shown in the middle of the operator's screen; and the control panel for adjusting stimulation current and information on the trial number are displayed on the right side. We first measured MVCs to select the level of background EMG activity that each participant was asked to maintain (typically between 5-15% of EMG during MVC) prior to H-reflex elicitation. To perform an MVC, the participant flexed the right wrist as strongly as possible for 5 s. This was repeated three times with ~60 sec between trials. The MVC of each trial was then calculated as the full-wave rectified voltage of an ~3 s time window in the middle of each trial. Final MVC values are the average of those three trials. Across the three participants, MVCs measured with the L-EES (MVC #1 – 0.78 ± 0.29 mV; MVC #2 – 0.70 ± 0.27 mV; measured from the L-EES electrode with the largest MVC) were similar to those measured using traditional electrodes (0.83 ± 0.14 mV). Fig. 5C shows an MVC sequence (i.e., three large bursts of EMG activity ~1 min apart) from participant HR-01, recorded with the L-EES (channel 11, electrode row

2, and column 3). MVC EMG was calculated as the average full-wave rectified voltage during the middle of each EMG burst (denoted with the dashed red lines; 3-5 s per flex). The low EMG level ~ 55-70 s after onset reflect a shift in the participant's position. Small postural adjustments by the participants are common, and do not affect the MVC analysis. In the case of the L-EES, EMG was recorded from the 16 electrodes simultaneously. Thus, the device allowed us to determine the electrode where the maximum EMG response was recorded. Fig. 5D shows EMG distribution across the electrodes during the MVC. EMG was maximum at channel 11 (row 2 and column 3). There was a technical problem that resulted in the loss of data from the first L-EES MVC recording for HR-02. Therefore, their MVC data is from an experiment performed on a different day. Supplementary Figs. S13–15 show additional MVC data from the participants. Next, we recorded recruitment curves (RCs) from each participant (Supplementary Figs. S16–21). RCs measure the H-reflex as a function of the stimulation current. Fig. 5E shows an RC (including M-wave and H-reflex sizes) for participant HR-01 from channel 11 (row 2 and column 3). M-wave and H-reflex latencies (i.e., timing) varies across people due to variables such as height (Burke 2016). For the Fig. 5E data, M-wave size was calculated as peak-to-peak EMG amplitude within a 6.2 to 15.9-ms window after stimulation; H-reflex size was calculated as peak-to-peak EMG amplitude in an 18.5 to 31-ms window after stimulation. Across the participants, average M-max sizes for the L-EES (16.33 ± 3.35 mV) were similar to those for the traditional electrodes (16.27 ± 4.82 mV); and the L-EES RC shapes were quite typical. As stimulation current increased, the H-reflex was elicited first; then the M-wave appeared at higher stimulation currents (for a detailed explanation see (Pierrot-Deseilligny and Burke 2005)). This occurs because the axon diameter of the largest Ia afferents is greater than that of the largest α -motoneurons, and the largest axons have the lowest threshold to electrical stimulation. As stimulation levels increase further, the size of the M-wave increase monotonically, while the H-reflex decreases in size due to antidromic collision of the reflex-mediated response with the direct motor response that produces the M-wave (Palmieri et al. 2004). H-reflex elicitation trials comprised the final data collected (Supplementary Figs. S22–24). They were used to determine the participant's baseline H-reflex for a given M-wave size. In actual H-reflex conditioning, they are also used to quantify changes in the participant's H-reflex induced by subsequent operant conditioning (Thompson et al. 2009). The grey traces in Fig. 5F are the responses—both M-waves and H-reflexes—elicited during each trial. Two blocks of 50 trials each were collected from each participant. The black trace is the average of the individual trials. The grey- and pink-shaded areas denote the time windows of the M-wave the H-reflex, respectively. The coefficients of variation for the M-wave and H-reflex were nearly identical for the L-EES (M-wave - 0.22 ± 0.03 ; H-reflex - 0.27 ± 0.04) and traditional electrodes (M-wave - 0.21 ± 0.03 ; H-reflex - 0.27 ± 0.04). One of the challenges in H-reflex operant-conditioning is consistent placement of electrodes across sessions (Botter and Vieira 2017; Kuck et al. 2018; Pierrot-Deseilligny and Mazevet 2000). In the current paradigms, photographs and template maps are used to ensure accurate electrode placements. A more efficient and potentially more effective approach is to use a multi-electrode array (such as the L-EES) to automatically determine the correct location for recording the M-wave and H-reflex. To examine automatic electrode positioning using the L-EES, we created a grid of 20 recording locations. We placed the L-EES on the participant's arm above 16 of those recording locations (Fig. 6A grid in upper left; Grid Position #1) and recorded reflex

responses. We then moved the L-EES ~1.5 cm laterally so that it was positioned above a different 16 recording locations (Fig. 6B grid in upper right; Grid Position #2) and recorded reflex responses again. The two heat maps in Figs. 6A and 6B show M-wave size as a function of recording electrode for each of these two positions. Waveforms from two channels in the grid appear in Fig. 6C and 6D for Grid Positions #1 (channel 14; row 1, column 2) and #2 (channel 12; row 1, column 2), respectively. The L-EES captures the shift in the distribution of the M-wave. These data suggest that a grid electrode could be used to detect the location of the maximum M-wave and reduce the need to precisely place electrodes at the onset of each session; this could substantially accelerate the setup process. In addition, the L-EES assisted with machine learning will help both the patients and clinicians obtain the automatic detection of MVC and recruitment curves from new users in real-time. This will allow for a more robust positioning that can lead to precisely placed electrodes for various human subjects with different muscle sizes and locations. In the future, we will conduct the study in the L-EES coupled with the deep-learning system.

4. Conclusion

The newly-developed, soft, breathable, and stretchable L-EES presented here provides high-fidelity recording of EMG activity from the forearm and appears applicable to therapies based on operant conditioning of the H-reflex. The array of 16 stretchable nanomembrane electrodes on a strain-limiting fabric enables reliable recording of neuromuscular responses from a large area of the skin and reduces motion artifacts. The results demonstrate that the L-EES is breathable and biocompatible with human skin; thus, it may be particularly useful for long-term EMG recording and for use in patients whose skin is particularly sensitive to breakdown. In summary, the wearable L-EES represents a new generation of biosensors and bioelectronics that can enhance EMG recording capabilities across multiple applications and disciplines. Areas of future work include optimization of the L-EES (e.g., reducing electrode size, incorporating the reference electrode into the device) and in-depth study of the long-term performance of L-EES for H-reflex operant conditioning with stroke patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- This article introduces the newly developed large-area epidermal electronic system (L-EES) for an operant conditioning of the Hoffmann reflex.
- The soft material-enabled L-EES that avoids the use of bulky, rigid sensors offers negligible mechanical and thermal burdens to the wearer, while minimizing issues of motion artifact.
- The breathable electronic system provides a skin-compatibility for the electromyographic activities as well as a reliable recording of direct muscle responses and H-reflexes.
- The bioelectronics enabling the non-invasive and targeted therapy improves a movement in people with a spinal cord injury.

Fig. 1. Design, architecture, and applications of large-area epidermal electronic system (L-EES). A) Schematic illustration of multi-layers of L-EES, including the nanomembrane Au electrode, encapsulating polyimide (PI), elastomer, and a strain-limiting fabric. Inset shows the enlarged view of a stretchable single electrode design. B) Photo of a fabricated L-EES, gently placed on the skin (forearm). Inset captures the zoomed-in view of a portion of a single electrode in the array. C) Illustration showing the experimental setup to use the L-EES for recording EMG and H-reflex on the forearm. D) Diagram representing the two neuron, one synapse pathway composed of the Ia sensory afferent and the α -motoneuron. This pathway can also be influenced by descending signals from the brain. E) Representative EMG response following transcutaneous electrical stimulation of the median nerve. A stimulation artifact is evident at the same time as the stimulation, followed by the direct muscle response (M-wave) and then the H-reflex. F) Flow chart illustrating the pathway of the signals involved in the recording of the H-reflex (red boxes) and M-wave (blue boxes).

Fig. 2. Computational and experimental study of mechanical behavior.

(A) Set of stress-strain curves for three different substrates (*blue*: electrodes on elastomer and fabric, *red*: elastomer and fabric, and *black*: fabric only). (B) Computational modeling result of a stretchable electrode that studied the stress distribution of strain on the mesh structure, caused by tensile strains of up to 70%. (C) Optical microscope photos that capture the stretching behavior of a fabricated electrode at 70% uniaxial stretching without mechanical fracture. (D, E) Relative resistance change of the fabricated electrode upon cyclic loadings (100 times) with 70% uniaxial stretching (D); and the magnified view of the change of resistance (black) according to the strain change (red), showing negligible change of resistance (E). (F) Correlation analysis of the data obtained from (E), showing the linear fit between relative resistance and applied strain. (G-I) A series of photos that capture the mechanical compliance of a fabricated L-EES with stretching (G), twisting (H), and rolling (I) with hands.

Fig. 3. Biocompatibility study of L-EES via cytotoxicity evaluation.

(A, B) Photos (left) and IR thermographs (right) of a pair of conventional gel electrodes (A) and L-EES (B), mounted on the skin over 8 hours. Note the adverse effects (skin rash and increased skin temperature) of the gel electrode with tape on the skin. (C) Comparison of measured temperature profiles on the skin from the gel electrode (top) and L-EES (bottom), showing negligible temperature change with the L-EES on the skin due to the enhanced breathability.

Fig. 4. L-EES for a 16-channel EMG mapping and reusability test.

(A) Schematic illustration of 16 channels of a single L-EES. (B) 16-channel EMG data, measured on the forearm, during multiple gestures, including no activity (rest), closed hand, thumb flexion, middle and ring finger flexion, and wrist flexion/extension. (C) Photos comparing a set of 16 conventional gel electrodes (top) with a single L-EES, both mounted on the forearm. (D) Measured EMG signals with the L-EES during wrist flexion via multiple mountings and removals up to 10 times, showing a negligible change of the EMG SNR. The summary graph on the right clearly captures the major advantage of the L-EES in terms of reusability, compared to the gel electrode. The gel electrodes had no signal after the three times. Error bars show standard deviations from three EMG measurement ($n=3$).

Fig. 5. EMG monitoring during the H-reflex operant conditioning protocol.

(A) Experimental setup, including L-EES, reference electrode, and ground electrode. The padded metal structure helps to maintain the participant's posture. (B) Evoked Potential Operant Conditioning System (EPOCS) software interface, showing the present full-wave rectified level of EMG activity, feedback on the EMG waveforms (i.e., M-wave and H-reflex) elicited during the previous stimulation, the number of trials completed, and the stimulation current. (C) EMG data (subject HR-01; channel 11; row 2, column 3) during MVC#1 (i.e., before the L-EES was laterally shifted). Three large bursts are times when the

participant was asked to flex. The small burst of activity around 55 s is from a small movement and completely normal. (D) MVC size measured from all 16 electrodes in the L-EES. Higher row and column number electrodes are more distal and lateral respectively. Mean rectified voltage was calculated as the mean of the absolute value of the EMG data during each of the three MVC time windows. The values for each of the three time windows were then averaged to obtain a final MVC value. (E) M-wave (black) and H-reflex (red) amplitude measured during a recruitment curve (RC; channel 11; row 2, column 3; participant HR-01). (F) Stimulation waveforms elicited during an H-reflex trial (HrT) from the L-EES (both HrTs occurred after the L-EES was shifted) from channel 14 (row 1, column 2; subject HR-01). M-wave and H-reflex time windows were 6.2 to 15.9 ms and 18.5 to 31 ms respectively. Peak-to-peak (P2P) M-wave and H-reflex sizes were calculated as the maximum minus the minimum EMG voltage in these time windows.

Fig. 6. Visual representation of M-Wave activity at M-Max (as measured during recruitment curves (RCs)) for different L-EES positions on the forearm of subject HR-01. (A, B) Peak-to-peak (P2P; calculated as the maximum minus the minimum EMG voltage in the M- and H-windows) M-wave sizes measured using the L-EES before (A) (Grid Position #1) and after (Grid Position #2) a ~1.5 cm lateral shift of the device (B). (C-D) EMG traces showing two representative channels (solid line = channel 12; row 1, column 2 and dotted line = channel 14; row 1, column 3). (C) Before the shift the larger signal was clearly measured from channel 14 (row 1, column 3; dotted line). (D) After the shift (although the difference is small) the larger signal was measured from channel 12 (row 1, column 2; solid line). The same M- and H-windows (6.2 to 15.9 ms and 18.5 to 31 ms respectively) were used during both RCs. A 10 Hz high-pass filter was applied to all the data.