Long-term behavioral, electrophysiological, and neurochemical monitoring of the safety of an experimental antiepileptic implant, the muscimol-delivering Subdural Pharmacotherapy Device in monkeys

Laboratory investigation

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Object. The authors evaluated the extent to which the Subdural Pharmacotherapy Device (SPD), chronically implanted over the frontal cortex to perform periodic, localized muscimol-delivery/cerebrospinal fluid (CSF) removal cycles, affects overall behavior, motor performance, electroencephalography (EEG) activity, and blood and CSF neurochemistry in macaque monkeys.

Methods. Two monkeys were used to adjust methodology and 4 monkeys were subjected to comprehensive testing. Prior to surgery, the animals’ behavior in a large test chamber was monitored, and the motor skills required to remove food pellets from food ports located on the walls of the chamber were determined. The monkeys underwent implantation of the subdural and extracranial SPD units. The subdural unit, a silicone strip integrating EEG electrodes and fluid-exchange ports, was positioned over the right frontal cortex. The control unit included a battery-powered, microprocessor-regulated dual minipump and radiofrequency module secured to the cranium. After implantation, the SPD automatically performed periodic saline or muscimol (1.0 mM) deliveries at 12-hour intervals, alternating with local CSF removals at 6-hour intervals. The antiepileptic efficacy of this muscimol concentration was verified by demonstrating its ability to prevent focal acetylcholine-induced seizures. During SPD treatment, the monkeys’ behavior and motor performance were again monitored, and the power spectrum of their radiofrequency-transmitted EEG recordings was analyzed. Serum and CSF muscimol levels were measured with high-performance liquid chromatography electrochemical detection, and CSF protein levels were measured with turbidimetry.

Results. The SPD was well tolerated in all monkeys for up to 11 months. The behavioral study revealed that during both saline and muscimol SPD treatment, the monkeys could achieve the maximum motor performance of 40 food-pellet removals per session, as before surgery. The EEG study showed that local EEG power spectra were not affected by muscimol treatment with SPD. The neurochemical study demonstrated that the administration of 1.0 mM muscimol into the neocortical subarachnoid space led to no detectable levels of this compound in the blood and cisternal CSF, as measured 1–125 minutes after delivery. Total protein levels were within the normal range in the cisternal CSF, but protein levels in the cortical-site CSF were significantly higher than normal: 361 ± 81.6 mg/dl. Abrupt discontinuation of 3-month, periodic, subdural muscimol treatments induced withdrawal seizures, which could be completely prevented by gradually tapering off the subdural muscimol concentration from 1.0 mM to 0.12–0.03 mM over a period of 2 weeks. The monkeys’ general health and weight were maintained. Infection occurred only in one monkey 9 months after surgery.

Conclusions. Long-term, periodic, transmeningeal muscimol delivery with the SPD is essentially a safe procedure. If further improved and successfully adapted for use in humans, the SPD can be used for the treatment of intractable focal neocortical epilepsy affecting approximately 150,000 patients in the US.

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Key Words • subdural pharmacotherapy • muscimol • macaque monkey • frontal cortex • cerebrospinal fluid • electroencephalography • epilepsy

Abbreviations used in this paper: ACh = acetylcholine; AED = antiepileptic drug; CCD = charged-coupled device; EEG = electroencephalography; HPLC-ED = high-performance liquid chromatography electrochemical detection; RF = radiofrequency; SPD = Subdural Pharmacotherapy Device.

DRUGS delivered through the cranial subdural/subarachnoid space on the pia mater can diffuse into the underlying cerebral cortical tissue. 10,26 We and others have demonstrated that when this “transmeningeal” route of drug delivery is used, focal neocortical seizures can be prevented and/or terminated. 9,15,21,23,39,42
Despite its therapeutic potential, this phenomenon has not yet been translated into a treatment for intractable focal neocortical epilepsy, a condition that affects approximately 150,000 people in the US.\(^6,7\) One of the remaining roadblocks to moving this therapy forward is the determination of the safety of localized AED delivery into the cerebral cortex through the subdural/subarachnoid space. To assess this, we implanted monkeys with an experimental drug-delivery apparatus, the Subdural Pharmacotherapy Device, and used this apparatus to deliver muscimol into the frontal cortex, transmeningeally, for more than 6 months.

Muscimol is a GABA\(_A\) receptor agonist occurring in the mushroom *Amanita muscaria*.\(^{11,18}\) When delivered systemically, muscimol exerts CNS side effects, prohibiting its use for therapeutic purposes.\(^{11,18,41}\) However, the single delivery of 1.0–2.5 mM muscimol into the rat or monkey neocortex via the transmeningeal route can exert more powerful antiepileptic effects than clinically used AEDs\(^2\) and can leave the animal’s behavior intact.\(^21\) These favorable pharmacological properties of muscimol, along with its water solubility and stability in solution,\(^21\) made this compound the drug of choice for the SPD. However, the safety of long-term transmeningeal muscimol delivery into the neocortex has been unknown.

The concentration of muscimol for this safety study was 1.0 mM. To verify that this is a relevant, antiepileptic concentration, we tested its potential to prevent focal, frontal cortical ACh-induced seizures. High, nonphysiological concentrations of ACh are known to cause seizures in the neocortex,\(^{14,16}\) through the suppression of M-current, a muscarinic-receptor-modulated K\(^+\) channel also involved in benign familial neonatal convulsions.\(^{19,38}\) We selected this seizure model because it produces reversible seizures with predictable onsets and progression and is thus appropriate for intracranial AED tests. Acetylcholine-induced neocortical seizures develop rapidly and can last for an hour or longer unless treated locally with muscimol or other AEDs.\(^{21,25}\) Local saline and artificial CSF are ineffective in changing the course of these seizures.\(^{21,25}\)

The SPD, based on US patent 6,497,699,\(^{22}\) was developed at NYU Langone Medical Center/School of Medicine for the treatment of intractable focal neocortical epilepsy. Detailed descriptions of the concept\(^21\) and hardware\(^25\) of the device, as well as preliminary data on its histological impact,\(^23\) are available. Essentially, the SPD includes a subdural silicone strip equipped with EEG recording electrode contacts and fluid exchange ports as well as a control unit. Each fluid port in the SPD strip is suitable for both directing a drug solution into the subarachnoid space and directing CSF out of the drug-exposed area. Local CSF removal is necessary to wash out inflammatory host reaction cells and molecules (for example, fibroblasts and collagen) that otherwise would clog the fluid ports and block transmeningeal drug diffusion. The function of the recording electrodes is to provide feedback on the effects of the delivered AED and thus help to adjust the effective drug-delivery parameters. The control unit, a battery-powered, microprocessor-controlled dual minipump equipped with an RF communication module, regulates fluid movement and electrophysiological data acquisition through the subdural strip. According to our plan, this control unit will ultimately be a fully internalized system, implantable as a single, encased device, subcutaneously, in the patient’s chest or abdomen. However, for the present study the control unit was secured to the cranium in monkeys to allow us to readily replace the battery, refill the minipump, or empty the CSF reservoir, when necessary.

The purpose of this study was to evaluate the impact of long-term use of the muscimol-delivering SPD on overall safety, occurrence of adverse events, behavior, motor functions, EEG activity, and, to a limited extent, blood and CSF neurochemistry in macaque monkeys. Nonhuman primates were used to obtain information as relevant to human conditions as possible. We selected the frontal cortex for muscimol delivery. Frontal cortical epilepsy is typically associated with the most treatment-resistant seizures in humans.\(^2,3,7\) Should the SPD be proved safe and effective, it will likely be used first for the treatment of this type of intractable focal epilepsy. Accordingly, this preclinical work has been focused on frontal cortical–SPD interactions.

**Methods**

**Animals**

Six adult male bonnet macaques (Macaca radiata) were used. Their weights over the course of the study are listed in Table 1. The drug-delivery system became clogged in Monkey 1, as regular CSF removal had not yet been implemented in this animal. Monkey 6 underwent implantation a month before the writing of this report. Thus, comprehensive testing was performed in Monkeys 2–5. All experimental procedures adhered to the standards detailed in the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, National Research Council, Washington, DC, National Academy Press, 1996). The described protocol was approved by the Institutional Animal Care and Use Committees of NYU Langone Medical Center/School of Medicine and that of SUNY Downstate Medical Center. To promote interactions between the monkeys, their 123 × 91 × 82–cm (height by width by length) housing cages, compliant with USDA regulations, were placed close to each other. However, only one monkey was placed in each cage. All behavioral, electrophysiological, and neuropharmacological studies were performed while the monkeys moved freely in a large, 178 × 124 × 124–cm test chamber equipped with a CCD camera on the ceiling. Thus, at no time were the monkeys restrained in a primate chair.

**Hardware and Software for the SPD**

The subdural strip (DocXS Biomedical) is a 0.8- to 1.3-mm-thick, custom-made combination of an Ad-Tech subdural EEG electrode and a fluid-port-integrating silicone rubber strip with a total surface area of 21 × 18 mm. The control unit (Cygnus, LLC) comprises a custom-made dual minipump and a controller board for a microprocessor, a minipump driver circuit, operational amplifi-
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TABLE 1: Weights of monkeys during the course of the study

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>1st Date*</th>
<th>2nd Date</th>
<th>3rd Date</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>11/17:09: 5.3</td>
<td>3/17/10: 5.1</td>
<td>6/29/10: 5.1</td>
</tr>
<tr>
<td>2</td>
<td>3/17/10: 5.1</td>
<td>6/29/10: 5.0</td>
<td>9/17/10: 5.3</td>
</tr>
<tr>
<td>4</td>
<td>11/16/10: 4.4</td>
<td>12/2/10: 4.4</td>
<td>7/11/11: 5.0</td>
</tr>
<tr>
<td>5</td>
<td>4/12/11: 5.9</td>
<td>7/11/11: 5.8</td>
<td>8/5/11: 6.0</td>
</tr>
<tr>
<td>6</td>
<td>7/26/11: 10.9</td>
<td>8/5/11: 11.5</td>
<td>not weighed†</td>
</tr>
</tbody>
</table>

* Day of surgical SPD implantation.
† Monkey 6 underwent implantation only 1 month before the writing of this article.

Enders, and a custom-made RF communication module. The dual minipump consists of 2 units, each connected to a 2.5-ml silicone reservoir. One reservoir is filled with either saline or muscimol for delivery and the other serves as a CSF collection reservoir. However, the design of the minipump allowed us to use the CSF reservoir unit for delivery of a seizure-inducing agent, ACh, during pharmacological tests. The minipump has been programmed to produce a standard flow rate of 120 μl/minute. During the course of the study, the saline/muscimol reservoir was refilled and the CSF collection reservoir was emptied biweekly under ketamine sedation (10 mg/kg, intramuscularly). Sterile saline and muscimol solutions were used, with the latter sterilized by flowing through 0.2-μm pore-size Acrodisc syringe filters. The unique function of the SPD software is to control the dual minipump in such a way that it can periodically alternate saline or drug delivery with CSF removals (US patent, serial no. 12/868/890, pending).

Experimental Design

In Phase 1 we collected baseline (control) behavioral and neurochemical data before surgery. Phase 2 included the surgical implantation of the SPD and a 1- to 1.5-month postoperative recovery period. Phase 3, lasting for 1–2 months, was dedicated to monitoring behavioral performance and EEG activity during the SPD’s periodic subdural saline delivery. This produced an additional set of control data. The device was then switched to periodic muscimol delivery, initiating Phase 4, which served to establish the antiepileptic efficacy of 1.0 mM subdural muscimol. Phase 5 was used to monitor the effects of long-term (1- to 4-month) subdural treatment with 1.0 mM muscimol on the behavior, EEG activity, and blood/CSF neurochemistry. The sixth phase was not included in the original study design. This phase was added once the effects of abrupt discontinuation of long-term muscimol treatment were observed, prompting us to devise a prevention strategy.

Surgical Implantation of the SPD

Implantation of the SPD in the 6 monkeys was performed after induction of general anesthesia, as described.23 Once the adequate depth of anesthesia was achieved, the monkey was placed in a stereotactic apparatus. The scalp was incised, exposing the skull, and the stereotactic manipulator was used to mark the interaural line. A right frontoparietal craniotomy was made with a 25-mm long and 7-mm short diameter, centered 15 mm to the right of the midline and 8.0 mm posterior from the interaural line.29 The fluid port–integrating subdural strip was slipped under the dura, as shown in Fig. 1 upper, over the frontal cortex. The craniotomy was closed with a 1.5-mm-thick silicone rubber sheet secured to the skull with self-tapping screws and dental cement, with the electrode wires and fluid tubes protruding from the sealed area (Fig. 1 lower). Anchoring screws and one or more epidural reference electrodes were placed in the skull. In 5 animals a 7.0-mm-diameter left frontoparietal craniotomy was also made with its center 8.5 mm to the left of the midline and 8.0 mm posterior from the interaural line. This accommodated a single-contact EEG recording strip slipped under the dura over the left motor cortex. The craniotomy was closed as on the right side. Next, the electrode wires and the fluid tubes were connected to the SPD microcontroller and minipump, respectively, and this was followed by covering all externalized components with a rectangular protective cap secured to the skull. The microcontroller and the minipump were connected to the battery. The minipump initiated the fluid delivery/CSF removal cycles shown in Fig. 2, with saline as the delivered fluid. A 2- to 3-minute telemetry EEG recording, transmitted by the microcontroller’s RF module to an external PC, was obtained to verify the integrity of the recording system. The protective cap was then closed, the skin around the cap was approximated with staples, and Neosporin was applied to the wound. Finally, anesthesia was discontinued and the animal was transferred back to his home cage. Postoperative care lasted for 2 weeks, as described,25 followed by another 2- to 4-week period without systemic drug treatment. During this period the monkeys were in their home cages.

Establishment of Antiepileptic Efficacy

After 1–2 months of periodic subdural saline delivery by the SPD, the operation of the minipump was temporarily suspended in Monkeys 2–5. Each monkey was sedated with 10 mg/kg intramuscular ketamine, and the protective cap was opened to access the pump. The CSF reservoir of the minipump was removed and replaced with a reservoir containing 300 mM ACh. The saline reservoir of the pump was also removed and replaced with a reservoir containing 1.0 mM muscimol (muscimol HBr, Sigma-Aldrich). The minipump was then reconfigured to allow the delivery of ACh to induce seizures. Ten to 15 hours after the monkey’s full recovery from the ketamine effect, the animal was placed in the test chamber and telemetry EEG was started. After a 2-minute baseline recording, the minipump was remotely instructed to deliver 60–120 μl ACh solution through the subdural fluid port–integrating strip for focal seizure induction. The detected seizures were scored as follows: 1, focal EEG seizures; 2, focal EEG seizures associated with contralateral convulsions; 3, generalized EEG seizures; and 4, generalized
EEG seizures associated with contralateral or bilateral convulsions. During Score 4 seizures, 60–120 μl muscimol was delivered via the minipump in an attempt to stop the ongoing seizure. After this experiment, the ACh solution was removed, the CSF reservoir was reconnected, and the original configuration of the minipump was restored, and periodic muscimol delivery/CSF removal cycles commenced (Fig. 2). One to 2 months later, the ACh experiment was repeated, with the ACh solution delivered in between 2 consecutive muscimol applications with the SPD pump. This tested whether periodic subdural treatment with 1.0 mM muscimol can prevent ACh-induced focal frontal cortical seizures and thus has antiepileptic properties. In each monkey, the same ACh dose was used during periodic saline treatment and during periodic muscimol treatment by the SPD.

**Behavioral Studies**

The behavioral studies were performed in Monkeys 3–5 before surgery (Phase 1), after surgery during periodic saline delivery by the SPD (Phase 3), and during the subsequent muscimol delivery by the device (Phase 4). This design served to determine whether SPD implantation and/or the muscimol delivery protocol induced motor deficits. Motor performance was monitored as follows. The animal was placed in the test chamber. In addition to the CCD camera, the chamber was also equipped with 8 food ports located on the walls at different spatial positions. Four of these ports were baited with food pellets, while the other food ports were empty. The task for the monkey was to explore the chamber (Fig. 3), climb to the food ports, and remove the hidden pellets from the 4 baited ports. This required unimpaired locomotion and hand skills. Each session consisted of 10 trials, with each trial allowing the monkey 5 minutes to remove the food pellets from all 4 baited ports. The next session was prompted by either the removal of all food pellets or the start of the 5th minute. The food pellets were delivered, and their effective removal by the monkey was detected, by a custom-made apparatus and software similar to what we used previously for squirrel monkeys. Thus, reaches into baited ports were counted for each session. This also yielded maximum food-pellet removals per session and average food-pellet removals per session values. To achieve maximum motor performance, the monkey had to remove 40 food pellets during each session. Using the CCD camera, the animals’ behavior was monitored on the screen of the PC. In most motor performance sessions, 20- to 60-second representative segments were stored for subsequent examination. For these functions, Pinnacle Dazzle Video Creator Plus USB video capture device and Pinnacle Studio 11 software were used. The video clip attached to this paper for online viewing was made with this system. Monitoring the monkeys’ behavior in the test chamber continued after the motor performance tests until the end of this study.

**Electroencephalography Studies**

The EEG studies, obtained in all monkeys, served to determine whether the implantation and long-term use of the subdural drug-delivery strip induced local neurophysiological abnormalities (for example, focal slowing, voltage depression, and interictal spikes) that might not be reflected in altered behavior. Acquisition of the EEG studies was performed by the SPD microcontroller, and the data were wirelessly transmitted by the SPD’s RF (Bluetooth) module to the PC, which was also used for behavioral data collection. These EEG recordings were collected while the monkey performed the behavioral task. Recording Channels 1–3 represented EEG activity at the ipsilateral (right), frontal cortical implantation site of the fluid port–integrating strip, while Recording Channel 4 represented EEG activity at the contralateral cortex (Fig. 4A). The contralateral cortex was implanted with only a single-contact subdural EEG electrode in Monkeys 1–5. Reference recordings with ×2000 gain, 0.1- to 70-Hz bandwidth, and 250-Hz analog-to-digital conversion sampling rate per channel were used, with epidural screw
or strip electrodes as reference electrodes. The EEG data were received with a Bluetooth transceiver, fed into the same PC that collected the behavioral data, and processed with custom-made software. This software performed 4-channel EEG acquisition, display, storage, and replay. The stored EEG files were both replayed for visual examination and subjected to Fast Fourier transform analysis to determine the power spectrum of the recordings, as described.\textsuperscript{21,23} For this analysis, 20-second EEG epochs were selected. The spectral intensities (power) of delta- (0.1–3.9 Hz), theta- (4.0–7.9 Hz), alpha- (8–11.9 Hz), beta- (12–29.9 Hz), and gamma- (30–70 Hz) frequency bands were calculated for these epochs. Only those recording segments were used during which the monkey 1) moved around in the test chamber and 2) did not eat. This assured that the EEG segments selected for power spectrum analysis were generated during similar behavioral states and were not confounded with chewing artifacts. Ipsilateral and contralateral recordings during periodic saline delivery and during periodic muscimol delivery were analyzed. The EEG data obtained in Monkeys 2–5 were processed for power spectrum analysis.

**Neurochemical Studies**

These studies included measurements of muscimol and total protein content in serum and CSF samples obtained in 4 monkeys. In Monkeys 2–4, samples were taken in the 1st week of periodic muscimol delivery, whereas in Monkey 5 the samples were taken in the 3rd week of periodic muscimol delivery. For each serum sample, 1 ml blood was collected from the saphenous vein, allowed to clot for 10 minutes, and then centrifuged to generate the sample for analysis. Collection times for the serum samples were 1, 10, 30, 60, and 120 minutes after delivery. The CSF samples included 0.4-ml samples collected from the cerebellomedullary cistern, with a percutaneous tap 5 and 125 minutes after subdural muscimol delivery. In addition, 0.2-ml fluid samples were collected from the

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**Fig. 2.** Temporal pattern of the fluid-delivery/fluid-removal cycles executed by the SPD dual minipump for up to 11 months. As indicated, one unit of the minipump directed either saline or muscimol into the frontal cortical subdural/subarachnoid space at 12-hour intervals, whereas the other pump unit removed CSF from the same site at 6-hour intervals to wash out accumulating products of host tissue reaction. Inflow and outflow volumes were adjusted to keep the total volume of the treated subdural/subarachnoid compartment constant. The extra flushing cycle, implemented for each 24-hour period, served to ensure adequate cleansing of the treated area. h = hours.

**Fig. 3.** Photograph of Monkey 4, taken while the animal explored the test chamber. Note the white protective cap on the animal’s head. This cap protected all externalized SPD components, including the minipump, the microcontroller, the RF communication module, and the battery, and could be opened to provide access to these components for maintenance. (Adaptation and encasement of these externalized SPD components for implantation in the chest or abdomen of human patients have yet to be accomplished.)
site of the fluid port–integrating subdural strip with the minipump, as the last step of the sampling protocol. During these procedures, the monkeys were sedated by intramuscular injection of 10 mg/kg ketamine. The samples were stored at −70°C until shipment.

Total protein levels in the CSF samples were determined at BioReliance, with standard turbidimetry methods using the COBAS Integra 400 plus system.

Muscimol measurements were performed as follows. Serum and CSF samples were stored at −80°C until processing time. Prior to HPLC-ED, the samples were thawed on ice, 5% perchloric acid was added to the sample, and the mixture was centrifuged at 4000 rpm for 5 minutes at 4°C. A 20-μl portion of the resulting supernatant was removed and analyzed for muscimol content by HPLC-ED. Muscimol serum and CSF samples (20 μl) were separated by HPLC-ED, consistent with isocratic separation methods used for amino acid analyses. The mobile phase consisted of 100 mM Na2HPO4, 22% MEOH, and 3.5% acetonitrile, pH 6.75, filtered through a 0.2-μm nylon membrane, degassed with 99% He for 20 minutes, and set to a flow rate of 0.7 ml/minute on a U3000 biocompatible isocratic pump (Dionex Corp.). Muscimol was detected by precolumn derivatization using a working solution of 2.2 mM O-phthalaldehyde (Pickering Labs) and 0.8 mM 2-mercaptoethanol (Sigma-Aldrich) mixed by automation with the sample at 10°C, 2 minutes prior to injection into the HPLC. Separation was achieved through a reversed-phase C18 column by Shiseido (Capcell MG, 4.6-mm inner diameter × 75-mm length, 3-μm pore size, with a C18 guard column attached) and electrically detected on a CouloChem III (Dionex Corp.) at the following potentials: E1, +250 mV; E2, +550 mV; and Guard, +650 mV at 45°C. Calibration curves were constructed using Chromeleon 6.8.0 software (Dionex Corp.), and the concentration (μg/ml) of muscimol for each sample was determined by comparing the peak area against the external standards. Serum and plasma samples were run in several batches, and new standard curves were generated with each corresponding sample batch for analysis. The detection limit of the assay was 0.05 μg/ml muscimol.

**Statistical Analysis**

The dependent variables for statistical analysis were:
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1) reaches into baited port counts, as obtained in the behavioral sessions, and 2) spectral intensity (power) values, as obtained in the EEG recordings. Natural log transformation was used for processing the EEG power values due to their skewed distribution. Both data sets were analyzed with SAS software (version 9.2, SAS Institute), using linear mixed models (ANOVA with random effects). For modeling the motor performance data, we used fixed-effects variables for 1) the 3 experimental phases corresponding to presurgery, postsurgery saline treatment, and subsequent muscimol treatment, and 2) the temporal order of the sessions within each phase. Random effects for animal within each phase were used in the modeling. For modeling of the EEG power data, we used fixed-effects variables for 1) the 5 examined frequency bands, 2) the 2 recording sites corresponding to Channels 1 and 4, and 3) the 2 treatment modalities corresponding to subdural saline and subdural muscimol administrations. Random effects for animal and frequency bands within an animal were used in the modeling. We used a heterogeneous variance model for both data sets. Separate variance error terms were considered for the different frequency bands in the EEG power data set and for the different phases in the motor performance data set.

Results

General Observations

All implant-treated monkeys maintained their weight over the course of the study—neither significant weight loss nor significant weight gain was observed (Table 1). Monkey 1, whose minipump became clogged in the 2nd postoperative month as no regular CSF removal procedure was yet implemented, carried the SPD for 9 more months without infection or deterioration of health. Monkey 2 remained healthy throughout his examination during 6 months of subdural saline delivery and 1 month of subdural muscimol delivery. Monkey 3 carried the fully functioning device and for 8 months underwent more than 30 minipump-refilling procedures without complications. In the 9th month Monkey 3 developed signs of infection, including lethargy, and was killed. Monkeys 4, 5, and 6 were healthy and in ongoing experiments at the time of the writing of this report, 10 months, 5 months, and 1 month after SPD implantation, respectively. Infection around the externalized SPD components did not develop. Such neurological symptoms as tremor, ataxia, paresis, or paralysis were absent in all animals. Daily monitoring of the movement pattern (Fig. 3), social interactions, and external features (for example, fur and eyes) of the implanted monkeys confirmed that the animals remained healthy during the entire study period (with the exception of the aforementioned case in Monkey 3).

Antiepileptic Efficacy of the SPD

Delivering 300 mM ACh into the site of the subdural fluid—integrating strip consistently induced focal EEG seizures (Fig. 4B). These were Score 1 seizures, which generalized within 4–8 minutes, producing contralateral clonic convulsions (Score 4 seizures). The ACh-induced seizures were terminated within 4–14 minutes following the delivery of 60–120 μl of 1.0 mM muscimol into the acute ACh seizure focus (Fig. 4C). Consistent with our prior power spectrum analyses, the detected EEG seizures were characterized with robust increases in the power of delta-, theta-, alpha-, and beta-frequency bands. This phenomenon of EEG power increase was used to capture the evolution of electrographic seizures in the course of long (≥ 30-minute) recording sessions (Fig. 5 upper). When periodic saline delivery with the SPD was switched to the periodic delivery of 1.0 mM muscimol for 3 weeks, focal ACh seizures were fully prevented. This was reflected in the lack of power increase in the EEG waves (Fig. 5 lower) and in the monkeys’ normal behavior. Establishment of the seizure-preventing, thus antiepileptic, efficacy of a 1.0-mM subdural muscimol dose was verified in all monkeys subjected to this test (Monkeys 2–5), justifying the use of this muscimol concentration in the subsequent safety studies.

Behavioral and Motor Functions

The behavioral task was performed by all tested monkeys before and after surgery, indicating their maintained motivation to explore. Table 2 summarizes the main motor performance data. The animals’ ability to remove all 40 food pellets from the baited ports within a session (maximum food-pellet removal per session) was unchanged throughout the study, regardless of whether this motor test was conducted before or after SPD implantation. After surgery, the minimum food-pellet removal per session value decreased during saline treatment but approached the presurgical level during the subsequent muscimol treatment. The mean food-pellet removal per session values remained similar throughout the study, varying between 37.9 ± 0.8 (mean ± SEM) and 39.9 ± 0.1, except in the postsurgical saline-delivery phase in Monkey 4, which yielded a value of 32.0 ± 2.4 (Table 2). Statistical analysis revealed that the mean number of reaches into baited ports in the 3 monkeys was 39.75 (95% CI 39.6–39.9) in the 12th session of the presurgical phase and 38.64 (95% CI 37.4–39.8) in the 12th session of the postsurgical muscimol-delivery phase. The difference was not statistically different (t6 = 1.82, p = 0.07). A video clip, presented in the online version of this report, shows a left-handed monkey moving around in the test chamber and skillfully removing food pellets from the baited food ports (Video 1).

Video 1. Behavior of Monkey 3, a left-handed animal, implanted with the SPD over the right frontal cortex. This recording was made 8 months after SPD implantation and 6 months after the start of periodic (12-hour) subdural muscimol deliveries alternating with periodic (6-hour) local CSF removals. The monkey approaches only the 4 baited food ports and skillfully removes the piece of food hidden in each of these ports. Note the normal locomotion and use of the left hand without any impairment. Click here to view with Media Player. Click here to view with Quicktime.

Electroencephalography Activity in the Subdural Implantation Sites

Wireless EEG recordings from the frontal cortical
drug-delivery/CSF-removal site were collected from all 5 monkeys for up to 11 months. We recorded no focal slowing, EEG flattening, loss of recording via 1 or more channels, or movement-related artifacts. Slight hemispheric electrographic differences, captured in the ipsi- and contralateral recording channels (Fig. 4A) occurred.

Results of statistical analysis of the power of delta, theta, alpha, beta, and gamma waves in the ipsilateral saline/drug-delivery site and the contralateral cortex, during both subdural saline and subdural muscimol delivery, are presented in Table 3. As expected, the mean power values of the 5 frequency bands differed significantly, regardless

![Graph showing EEG seizure and power changes](image)

**TABLE 2: Motor performance measures in 3 monkeys before and after SPD implantation**

<table>
<thead>
<tr>
<th>Motor Performance Measures</th>
<th>Experimental Phase</th>
<th>Monkey 3</th>
<th>Monkey 4</th>
<th>Monkey 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>max/min food-pellet removals/session</td>
<td>preop</td>
<td>40/38</td>
<td>40/35</td>
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<td></td>
<td>postop: SPD periodically delivers saline</td>
<td>40/12</td>
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<tr>
<td></td>
<td>after periodic saline treatment: SPD periodically delivers muscimol</td>
<td>40/25</td>
<td>40/29</td>
<td>40/35</td>
</tr>
<tr>
<td>mean ± SEM food-pellet removals/session</td>
<td>preop</td>
<td>39.9 ± 0.1</td>
<td>39.8 ± 0.2</td>
<td>39.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>postop: SPD periodically delivers saline</td>
<td>36.5 ± 2.3</td>
<td>32.0 ± 2.4</td>
<td>38.5 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>after periodic saline treatment: SPD periodically delivers muscimol</td>
<td>38.5 ± 1.0</td>
<td>37.9 ± 0.8</td>
<td>39.5 ± 0.4</td>
</tr>
</tbody>
</table>
Safety of the Subdural Pharmacotherapy Device in monkeys

<table>
<thead>
<tr>
<th>Subdural Treatment</th>
<th>EEG Band</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Delta</td>
</tr>
<tr>
<td>saline delivery site (a)</td>
<td>136.3 ± 16.5</td>
</tr>
<tr>
<td>contralateral site (b)</td>
<td>107.7 ± 13.6</td>
</tr>
<tr>
<td>muscimol delivery site (c)</td>
<td>160.1 ± 21.1</td>
</tr>
<tr>
<td>contralateral site (d)</td>
<td>99.2 ± 21.1</td>
</tr>
<tr>
<td>p values</td>
<td></td>
</tr>
<tr>
<td>a vs b</td>
<td>0.225</td>
</tr>
<tr>
<td>a vs c</td>
<td>0.464</td>
</tr>
<tr>
<td>c vs d</td>
<td>0.009†</td>
</tr>
</tbody>
</table>

* Raw data (mean ± SEM) are shown. Probability values, derived from a linear mixed model using log-transformed power values, were not adjusted for multiple testing.
† Difference due to the larger delta band in the delivery site than in the contralateral site.

of recording site or treatment (F 4,12 = 0.024, p = 0.877), were not significant.

### Muscimol and Protein Concentrations in Blood and CSF

Retention time and peak for muscimol were identified in samples containing 1.0 mM muscimol dissolved in saline at pH 7.2 to 7.4 (Fig. 6A). Analysis of blood (serum) samples collected in Monkeys 3 and 4 before SPD implantation, and thus before any exposure to muscimol, confirmed the lack of muscimol peak in these samples (Fig. 6B). Analysis of serum samples collected 1, 5, 10, 30, 60, and 120 minutes after the single subdural delivery of 40 μl of 1.0 mM muscimol showed that the levels of this drug were below the detection limit of 0.05 μg/ml in 13 of the 15 samples (Fig. 6C, Table 4). Similar data were found in Monkey 2, used for methodological adjustments: no muscimol was detected in the serum at any time after the single application of 40 μl of 1.0 mM subdural muscimol. Examination of muscimol concentrations in cisternal CSF samples consistently showed that the molecule could not be detected in this medium either, regardless of whether the samples were taken 5 or 125 minutes after subdural muscimol delivery (Table 4). The same results were obtained with cisternal CSF samples from Monkey 2. When serum and CSF samples were collected 35–45 minutes after the subdural application of muscimol to attempt to stop an ongoing seizure in Monkeys 3, 4, and 5, muscimol was again absent in the collected blood and cisternal CSF samples (Fig. 4D and E). However, analysis of the CSF collected from the subdural ACh/muscimol-delivery (implantation) site in the 3 animals revealed muscimol concentrations of 42.2, 27.1, and 21.5 μg/ml (30.7 ± 6.1 μg/ml), respectively (Table 4). Figure 4F shows the chromatogram of a CSF sample collected from the implantation site following localized muscimol delivery during an ongoing ACh seizure. Only these samples from the implantation site contained muscimol in measurable concentrations. In summary, muscimol concentrations in the systemic blood and CSF circulation after a single subdural muscimol delivery with the SPD were below the detection level in 30 of 32 samples collected in 4 monkeys, regardless of whether these sample collections took place 1 week or 3 weeks after the start of periodic subdural muscimol treatment or whether the samples were collected in seizure-free or postseizure periods.

The total protein level in the systemic CSF circulation, as measured in the cisternal CSF samples, was in the normal range, 30.6 ± 11.7 mg/dl. However, a significantly increased total protein concentration, 361.4 ± 81.6 mg/dl was detected in the CSF samples collected from the subdural implantation/drug-delivery site (p = 0.035, paired t-test).

### Withdrawal Seizures and Their Prevention

No spontaneous epileptiform EEG or behavioral signs occurred in Monkeys 1 and 2. Following termination of subdural muscimol delivery, withdrawal seizures developed in Monkeys 3 and 4. These seizures occurred within a day after abrupt discontinuation of 3 months of periodic subdural muscimol delivery (Fig. 7). However, a single administration of 1.0 mM muscimol with the SPD promptly stopped the seizures and normalized both EEG activity and behavior (Fig. 7). When we subsequently reduced the concentration of the applied muscimol in a gradual fashion, from 1.0 mM to 0.12–0.03 mM over a period of 2 weeks, discontinuation of muscimol delivery did not lead to epileptiform EEG or behavioral events (Fig. 7). Once withdrawal seizures were prevented with this protocol, epileptiform EEG or behavioral signs did not recur.
Discussion

The use of intracranial drug-delivery devices for treatment of localized brain disorders is a promising neurosurgical strategy that has emerged over the past 10–15 years. The present study addressed, for the first time, the most basic issues regarding the safety of long-term drug delivery into the cerebral cortex transmeningeally, via the subdural/subarachnoid space. The device used for this study was the SPD, a nonrespon-

sive, automatic “hybrid neuroprosthesis,” designed to pe-

riodically administer muscimol into neocortical seizure foci to prevent seizures. Treatment-resistant epilepsy has a high morbidity and mortality rate, approaching 10% over a decade in some cohorts. Focal drug delivery offers multiple advantages in these patients, including the ability to use drugs that may not be systemically tolerable or able to cross the blood-brain barrier, delivery of high concentrations of the drug in the affected neocortical area, and minimal or no drug levels in systemic tissue or other subcortical and cortical brain areas in which a drug would cause adverse effects.

Functioning SPD Implants Carried by Nonhuman Primates Without Adverse Effects on Health for Nearly 1 Year

The SPD can be safely implanted and used in ma-

caque monkeys for nearly 1 year. The animals maintained their presurgical weight throughout the study, indicating that their eating habits and general health were not af-

fected by SPD implantation and use. Signs of pain in nonhuman primates, such as refusal of food and water, sitting in a crouched posture, or lack of motivation to perform behavioral tasks, were absent. The ability to study the monkeys’ behavioral performance without difficulty for months after implantation indicates the safety of this treatment. Infection occurred in 1 of 6 monkeys after 8 months of involvement in the experiments. We are aware of the risk of infection caused by repeated refilling of the drug-delivery pump reservoir through externalized tubing. This is a problem that can be eliminated once the device is fully implanted in the body and refilled percu-

taneously.

Frontal Cortical Muscimol Delivery With the SPD Causes no Local EEG Abnormalities and Leaves Behavior and Motor Functions Intact

A key finding was that delivery of 1.0 mM muscimol into the frontal cortical subdural/subarachnoid space, alternating with periodic local CSF removals, did not affect local EEG activity and did not interfere with normal behavior. The power spectrum of frontal cortical EEG waves during periodic muscimol treatment remained in the physiological range, and the behavior-induced modulation of EEG activity was unaffected by localized mus-

cimol administration. The sole alteration of slightly de-

creased delta power in the contralateral site was not due to muscimol but to the implant itself, possibly due to pres-

sure by the roughly 1-mm thick subdural strip. This effect is unlikely in humans in whom the subdural/subarachnoid space is wider. During surgery, the drug-delivery/CSF-removal strip was placed over the right motor cortex. Accordingly, significant damages in these areas produced by implantation, muscimol delivery, and/or local CSF re-

moval should have been associated with signs of motor deficit. No such impairments were observed: Video 1 (on-

line version) proves this conclusion. After surgery, we did observe a temporary, slight decrease in the food-pellet...
removals per session. We think this was not a reflection of a motor deficit but, rather, was related to the monkeys’ re-habituation of the test chamber, which they did not visit during the 1- to 1.5-month postoperative period.

**Negligible Risk of Systemic Muscimol Exposure During SPD Treatment**

A critical component for successful SPD drug delivery to discrete neocortical areas is preventing the drug from leaking beyond local cortex through more widespread CSF or vascular circulations. More widespread distribution could lead to greater CNS toxicity (for example, effects on hypothalamus or brainstem) or systemic toxicity. Leakage could result from hardware failure, very high drug concentrations, and neurobiological factors, such as increased blood-brain barrier permeability after seizures. The risk of leakage can be determined by monitoring cisternal CSF and serum drug levels during subdural treatment. Our measures, using HPLC-ED, revealed undetectable serum and cisternal CSF muscimol concentrations at various times after subdural delivery of 1.0 mM of muscimol. Measurements were made both in

**TABLE 4: Muscimol concentrations in blood and CSF samples in monkeys subjected to subdural muscimol delivery by the SPD**

<table>
<thead>
<tr>
<th>Sampling Condition</th>
<th>Sampled Medium</th>
<th>Time From Muscimol Delivery to Sampling (mins)</th>
<th>Muscimol Concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>samples collected after 40 μl of 195 μg/ml (1.0 mM) muscimol delivered via SPD, w/o preceding seizure induction</td>
<td>blood</td>
<td>1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>blood</td>
<td>10</td>
<td>&lt;0.05 0.45</td>
</tr>
<tr>
<td></td>
<td>blood</td>
<td>30</td>
<td>&lt;0.05 0.30</td>
</tr>
<tr>
<td></td>
<td>blood</td>
<td>60</td>
<td>&lt;0.05 0.05</td>
</tr>
<tr>
<td></td>
<td>blood</td>
<td>120</td>
<td>&lt;0.05 0.05</td>
</tr>
<tr>
<td></td>
<td>cisternal CSF</td>
<td>5</td>
<td>&lt;0.05 0.05</td>
</tr>
<tr>
<td></td>
<td>cisternal CSF</td>
<td>125</td>
<td>&lt;0.05 0.05</td>
</tr>
<tr>
<td>samples collected after 60–120 μl of 195 μg/ml (1.0 mM) muscimol delivered via SPD to stop an ongoing focal seizure</td>
<td>blood</td>
<td>35–45</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>cisternal CSF</td>
<td>35–45</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>delivery site CSF</td>
<td>35–45</td>
<td>42.2 27.1 21.5</td>
</tr>
</tbody>
</table>

* The HPLC-ED data generated with the same methodology are included in the table.
seizure-free conditions and after focal seizure induction followed by subdural muscimol delivery. When muscimol was measured in serum and cisternal CSF 35–45 minutes after a focal seizure, the drug levels were below the detection threshold. As expected, muscimol levels in the subdural implantation-site CSF were detected after SPD delivery. Muscimol metabolites were not monitored in this study, and thus we cannot exclude with certainty that such metabolic products accumulated in the systemic circulation. However, HPLC-ED assays of serum samples collected from the monkeys before any muscimol exposure (Fig. 6B) yielded the same chromatography peaks as those 120 minutes after muscimol exposure (Fig. 6C). Since no new peaks were observed, it is likely that no muscimol metabolites were present in the blood after muscimol delivery with the SPD, as muscimol in blood is completely metabolized within 120 minutes. The HPLC-ED data of this study are consistent with the very low doses of muscimol applied with our subdural delivery method. Thus, the delivery of 40 μl of 1.0 mM muscimol HBr (MW 195.0) equals a 7.8-μg dose of this compound.

Periodic Removal of Local Inflammatory Host Reaction Products Critical for Maintaining SPD Functions

All foreign bodies can induce an inflammatory reaction in the brain, including recording electrodes, stimulators, and other devices, without infection. Despite these reactions, intracranial shunts, reservoirs, and electrodes have been safely and effectively used for decades. Local inflammatory processes pose a specific problem for chronic drug-delivery implants because proliferating fibroblasts, gliosis, aggregating extracellular matrix proteins, and other processes can clog the system and impede drug diffusion into the target area. Side effects of antiinflammatory drugs limit their long-term use. We devised a strategy to reduce the effects of the inflammatory response: to periodically wash out local inflammatory cells and molecules from the fluid exchange ports. This strategy was effective in the present study to prevent clogging and to allow adequate transmeningeal drug diffusion into the cortical tissue with neuropharmacological responses. We found no adverse effects of this procedure.

Withdrawal Seizures After Abrupt Discontinuation of Long-Term Subdural Muscimol Treatment: Preventable Side Effect

The detection of withdrawal seizures after the abrupt cessation of subdural muscimol after 3 months of treatment confirms the paramount importance of long-term (>2-month) behavioral and electrophysiological monitoring in studies involving intracranial drug-delivery implants. This phenomenon was not observed in prior animal studies with muscimol, likely because these studies did not extend monitoring beyond 2–3 weeks. Importantly, gradual reduction of the muscimol dose prior to cessation prevented withdrawal. Thus, while increased seizure susceptibility does seem to develop upon abrupt discontinuation of chronic subdural muscimol, similarly to the increased seizure susceptibility that follows the abrupt discontinuation of oral AEDs, this potential side effect can be controlled. As a preemptive strategy, we are now modifying the SPD software so that it can instruct the minipump to start to deliver gradually decreasing doses of muscimol once 90% of the drug reservoir is emptied. This should prevent the risk of increased seizure susceptibility related to the abrupt discontinuation of chronic subdural muscimol treatment.

Potential Adaption of the SPD for Safe and Effective Prevention of Otherwise Untreatable Focal Neocortical Seizures in Humans

This study is the first among several from our laboratory that will hopefully advance the SPD toward clinical trials. Several other pieces of data are yet to be obtained, including information on whether transmeningeal muscimol penetrates all layers of the treated neocortex, whether long-term subdural muscimol treatment induces tolerance, whether the subdurally implanted device causes clinically relevant histological alterations in the interfaced neocortical tissue, and whether subdural muscimol treatment is as effective in controlling chronic (for example, alumina gel–induced) neocortical seizure foci such as acute foci in the present study. These questions need to be answered. Also, the SPD should be further miniaturized and properly encased to be fully implantable in the human body, and pathological studies are required to determine risks for systemic complications and teratogenic and/or carcinogenic side effects.

Nevertheless, this study did prove that essentially the muscimol-delivering, frontal cortical SPD is safe and effective in primates for at least about a year without hardware failure, muscimol leakage, or the induction of behavioral, motor, or neurophysiological abnormalities. Thus, based on these pieces of evidence, it appears that SPD treatment has the potential to provide seizure prevention to at least a subclass of patients who suffer from otherwise treatment-resistant, focal neocortical epilepsy who are not candidates for resective surgery. The present device is a nonresponsive hybrid neuroprosthesis that delivers the seizure-controlling drug periodically, in an automatic, not on-demand, fashion. We view the automatic SPD as a first-generation intracranial pharmacotherapy device, a basic platform on which responsive, on-demand drug-delivery systems can be built in the future for the treatment of other subclasses of focal epilepsy.

Technical and Methodological Novelties for General Neurosurgical Practice and Research

The wireless EEG recordings we obtained during behavior and convulsions were free of artifacts due to cable movements. Such artifacts often bedevil cable-based, diagnostic intracranial recordings. The described battery-powered microcontroller-RF communication apparatus could be readily adapted for human use, and such an apparatus could be placed in the postoperative head wrap without difficulty to transmit data for 24-hour video/EEG recordings. This method would improve diagnosis and allow the patients to move freely after the implantation of strip, grid, or depth electrodes.

The protective cap on the monkey’s head was critical in avoiding infection around the externalized implant components and thus performing wireless EEG record-
ing and remote-controlled intracranial drug delivery for nearly 1 year, in freely behaving monkeys, without the need of chair restraint. This methodology can significantly expand the possibilities of neurosurgery studies in nonhuman primates in a humane way.

Finally, this paper describes for the first time a method for measuring muscimol levels from blood or CSF samples with HPLC-ED. This method offers better sensitivity than HPLC-ultraviolet, it is less costly than gas chromatography and mass spectrometry, and it yields reliable data. As such, it is ideally suited for larger-scale muscimol assays in expanded preclinical tests and future clinical trials.

Conclusions

The major findings of this study were as follows. 1) The SPD can be readily implanted in monkeys, and the animals tolerate this device well for nearly 1 year. 2) Periodic local CSF removal from the treated neocortical subarachnoid space can effectively wash out inflammatory host reaction cells and proteins, preventing clogging and allowing adequate transmeningeal drug diffusion. 3) Periodic transmeningeal delivery of 1.0 mM muscimol into the frontal cortex exerts local antiepileptic effects. 4) Periodic frontal cortical muscimol applications, alternating with local CSF removals, can be maintained for many months without affecting motor functions or causing EEG abnormalities. 5) Delivering 1.0 mM muscimol into the neocortical subarachnoid space leads to no detectable levels of this compound in the systemic CSF and cardiovascular circulation. 6) Like abrupt discontinuation of chronic oral AED therapy, abrupt discontinuation of 3-month long, periodic subdural muscimol treatments with the SPD induces increased seizure susceptibility, a potential side effect that can be prevented by gradually tapering off the subdural muscimol concentration. Based on these findings in nonhuman primates, we conclude that long-term, periodic, transmeningeal muscimol delivery with the SPD is essentially a safe procedure. If the hardware and software of the device, as well as its surgical implantation and drug-delivery protocol, are further improved and successfully adapted for human use, the SPD may be used for the treatment of intractable focal neocortical epilepsy affecting about 150,000 patients in the US.

Disclosure

This work was supported by the Epilepsy Research Foundation (grant no. 140929 to N.L.) and funds from Finding A Cure for Epilepsy & Seizure (FACES). Dr. Ludvig holds, with Lorant Kovacs, US patent no. 6,497,699, on which the SPD is based. This patent is not yet licensed, and Dr. Ludvig is not associated with any business entity, nor did he receive any payment or promise of payment during the course of this study other than his academic salary. Mr. Medveczky is a consultant for Cygnus, LLC. Dr. Devinsky is a patent holder at NYU Medical Center.

Author contributions to the study and manuscript preparation include the following. Concept and design: Ludvig, Devinsky, French, Carlson, Kuzniecky. Acquisition of data: Ludvig, Tang, Baptiste, Vaynberg, Vazquez-DeRose. Analysis and interpretation of data: Ludvig. Drafting the article: Ludvig. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Ludvig. Statistical analysis: Ludvig, Stefanov. Administrative/technical/material support: Ludvig, Kuzniecky. Study supervision: Ludvig. Principal investigator: Ludvig. Construction of the SPD for the study: Medveczky.

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