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A fast intracortical brain-machine interface with patterned optogenetic feedback

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Abstract

Objective. The development of brain-machine interfaces (BMIs) brings a new perspective to patients with a loss of autonomy. By combining online recordings of brain activity with a decoding algorithm, patients can learn to control a robotic arm in order to perform simple actions. However, in contrast to the vast amounts of somatosensory information channeled by limbs to the brain, current BMIs are devoid of touch and force sensors. Patients must therefore rely solely on vision and audition, which are maladapted to the control of a prosthesis. In contrast, in a healthy limb, somatosensory inputs alone can efficiently guide the handling of a fragile object, or ensure a smooth trajectory. We have developed a BMI in the mouse that includes a rich artificial somatosensory-like cortical feedback.

Approach. Our setup includes online recordings of the activity of multiple neurons in the whisker primary motor cortex (vM1), and delivers feedback simultaneously via a low-latency, high-refresh rate and spatially structured photo-stimulation of the whisker primary somatosensory cortex (vS1), based on a mapping obtained by intrinsic imaging.

Main results. We demonstrate the operation of the loop and show that mice can detect the neuronal spiking in vS1 triggered by the photo-stimulations. Finally, we show that the mice can learn a behavioral task relying only on the artificial inputs and outputs of the closed-loop BMI.

Significance. This is the first motor BMI that includes a short-latency, intracortical, somatosensory-like feedback. It will be a useful platform to discover efficient cortical feedback schemes towards future human BMI applications.

1. Introduction

Invasive BMIs have recently transitioned from research prototypes (Fetz 1969, Chapin et al 1999, Wessberg et al 2000) to the first trials in human patients (Hochberg et al 2006, Hochberg et al 2012, Collinger et al 2013, Wodlinger et al 2015, Ajiboye et al 2017). These trials show that over the course of months, a fully paralyzed patient can learn to control a robotic prosthesis and perform simple actions such as to grasp a bottle and drink from a straw. These achievements rely solely on the readout of the spiking activity from electrodes chronically implanted in motor areas, which is translated into motor commands for the prosthetic device.

Compared to the physiological condition, almost all artificial prosthetic devices are devoid of proprioceptive and somatosensory readouts, despite their critical role in precision movements (Akay et al 2014, Sainburg et al 1995) and grip (Monzée et al 2003, Johansson et al 1984) as well as to achieve embodiment of the prosthesis (Marasco et al 2011).
Prostheses that address sensory periphery deficits predate motor BMIs and include cochlear (Djourno et al. 1953), retinal (Brandli et al. 2016) and tactile implants (Clippinger et al. 1974, Raspopovic et al. 2014, Ortiz-Catalan et al. 2014, Graczyk et al. 2016, Oddo et al. 2016). Prostheses mediated by cortical microstimulation are rarer but they are increasingly successful. Prototypes have been developed to rescue vision (Brindley et al. 1968, Dobelle et al. 1974, Dobelle et al. 2000) and an active effort is underway to build a somatosensory prosthesis based on S1 microstimulations (Flesher et al. 2016, O’Doherty et al. 2009, O'Doherty et al. 2011). Such S1-targeting cortical prosthesis allows spatial discrimination between touch-like stimuli (Tabot et al. 2013). It can also be used to support a redundant, population vector coding (Dadarlat et al. 2015) of touch-like inputs, as well as other, non-touch related, but topically structured S1 microstimulation-based inputs (Hartmann et al. 2016). Still, there is a debate regarding the benefit of multichannel feedbacks compared to single channel feedback (Kim et al. 2015a).

One attractive feature of S1 is the topographical representations of touch inputs at the surface of the cortex (Penfield et al. 1937). This homology between the spatial organization of the sensory periphery and of the cortical surface is a core computational principle of cortical circuits and it constrains sensory perception (Estebanez et al. 2018). Therefore, a feedback based on spatial patterns of cortical stimulation may be both intuitive and carry a larger set of information compared to recent feedback strategies based on time-dependent microstimulations in S1 (O’Doherty et al. 2009, O’Doherty et al. 2011).

However, despite the efforts to design attractive multi-site S1 microstimulations, so far they have not been integrated into closed-loop BMIs. More generally, few studies have integrated an artificial feedback into a closed-loop invasive BMI (O’Doherty et al. 2011, Prsa et al. 2017). This is in part because electrical microstimulations in S1 disrupt the readout of electrophysiological inputs in M1 and therefore prevent continuous closed-loop operation (O’Doherty et al. 2011). In addition, reproducing a somatotopy can be technically challenging since the spatial resolution of current epi-cortical microstimulation arrays is limited to a pitch of at least several hundreds of microns between adjacent electrodes (Flesher et al. 2016) and the actual spatial extent of electrical microstimulation is hard to control and may depend on the specific axonal connectivity of the stimulated area (Histed et al. 2009). Finally, electrical stimulations are not selective to the neuronal cell type and activate both excitatory and inhibitory neurons at the same time.

In contrast, optogenetic BMI feedback would not be affected by these shortcomings (Prsa et al. 2017). We therefore developed a BMI prototype in the head-fixed mouse model that couples into a closed BMI loop both a readout of neuronal activity in vM1 using chronic extracellular recording electrodes and a feedback delivered through the spatially patterned, low latency photostimulation of Channelrhodopsin2-expressing pyramidal neurons in vS1. We characterize this loop, and show that the vS1 artificial stimulation triggers controlled spiking activity. We show that this activity can be detected by the mouse in the context of a detection task, and finally we show that the mouse can learn to carry a task exclusively through the inputs and outputs provided by the closed-loop BMI.

2. Materials and methods

2.1. General structure of the bidirectional BMI

To setup this BMI with feedback, we have worked on a number of modules that we describe and test (Figure 1). First a photostimulation system capable of dynamically updating, within
milliseconds, 100 µm scale illumination patterns at the surface of vS1 cortex (Section 2.2). Second, an intrinsic imaging device coupled to the photostimulation system to target the photostimulations at functionally localized barrels of vS1 (Sections 2.3 to 2.6). Third, an extracellular recording system that acquires neuronal activity from vM1 (Sections 2.7, 2.8). Fourth, a computer software that connects the input and output modules (Sections 2.9, 2.10), and delivers timely water-based rewards (Section 2.11). The code as well as a list of the parts used to build the setup has been made available at the following link: https://github.com/BMIunic/Sensorimotor_BMI.git.

2.2. Photostimulation optical sub-system

To project photostimulation patterns into vS1, we used a Digital Light Processing module (DLP, Vialux V-7001, Germany) containing a 1024 x 768 Texas Instruments micro-mirror chip, which was illuminated by a 462 nm blue LED (optimal to photoactivate Channelrhodopsin, Bamann et al 2008). This device (Figure 1B) allowed us to display arbitrary patterns of stimulation by streaming binary (black and white) frames through a USB 3.0 link using the manufacturer API.

We coupled optically the DLP to a tandem-lens macroscope that focuses the divergent light beam that comes out of the projector and projects it on the surface of the cortex of a head-fixed mouse. Two lenses were arranged in an L-shape optical pathway with a dichroic beamsplitter in between. Light patterns entered the macroscope though the rear end of the first lens (150 mm smc Pentax-A 645 lens) and went through the dichroic beamsplitter placed at a 45° angle. We used camera lenses instead of optical lenses to minimize image distortions (Figure 1C,D). The beamsplitter (640nm cut off wavelength; 60x60 mm; Semrock) reflected the light patterns to the front end of the second lens (50 mm Nikon NIKKOR lens), which demagnified the image by a factor of 3 and projected it over the cortex of a mouse. The working distance of the macroscope is 4.6 cm.

2.3. Intrinsic imaging optical sub-system

We combined this projection pathway with a secondary “measurement” tandem-lens optical pathway where the light from a red or green LED, illuminating the cortex, is reflected back through the rear end of the second lens and transmitted by the beamsplitter to the front end of a third lens (50 mm Fujinon HF50HA-1B). This lens was mounted on a 12-bit monochrome CCD camera (Basler aCA640-120um). This second optical pathway was devoted to the acquisition of intrinsic signals from the cortex (Figure 2).

2.4. Implantation and preparation for intrinsic imaging

All animal experiment procedures were in accordance with European and French law, and CNRS guidelines. Initial surgery was carried under Isoflurane anesthesia (1-4% Isoflurane depending on mouse state, assessed by breathing rate and response to tail pinch), in 100% O2. The surgery was performed in a stereotactic frame (Stoelting) on a regulated heating pad, with the mouse held by a nose clamp. The scalp was resected and conjunctive tissues were removed. At this stage, a head fixation bar was attached (Figure 1E) using a bonding layer of cyanoacrylate glue applied to the skull (Henkel Loctite) followed by dental cement (Lang Dental, USA) to encase the attachment bar (Guo et al 2014).

After this initial surgery, the animal was transferred under the macroscope (Figure 1B) to identify the location of the cortical columns that receive the input projection from individual whiskers in vS1 (Figure 2B). The mouse underwent a first intrinsic imaging session (Figure 2C) through the intact skull (Figure 2D-F), in order to identify functionally
the location of the cortical area that responds to whisker deflections, and more specifically the
column ("barrel") that corresponds to the central vibrissa of the whiskerpad, the C2 whisker
(Petersen et al 2007).

2.5. Intrinsic imaging procedure over vS1

To carry intrinsic imaging (Grinvald et al 1986) and identify the location of the barrel
associated with a specific whisker (Knutsen et al 2016), a red LED (625 nm) was used to
illuminate vS1 through the skull of the isofurane anesthetized mouse (Figure 2C). To
increase as much as possibility the skull transparency, a temporary well made-up of high
vacuum grease (Dow Corning, USA) was built around vS1 (coordinates P-1.5mm L-3.3mm
from bregma, Figure 1E), filled with Ringer solution and topped with a coverslip (Fisher
Scientific, USA). This configuration ensured good light transmission from cortex to the
camera sensor, in particular through the skull. The CCD camera (Figure 2C) was triggered to
acquire 659 x 494 px images at a rate of 50 fps. 2 s after the onset of the imaging, the C2
whisker (Figure 2A) was deflected by a piezoelectric bender (PI PICMA Bender) mounted on
a vibration-dampening holder (Jacob et al 2010) (Figure 2C). The whisker was deflected 100
times at 100 Hz following a train of 5 ms square wave deflections in the caudorostral axis
(Figure 2D).

This acquisition sequence was repeated 20 times to obtain a trial-averaged movie. To
to identify the location of the brain region activated by the stimulated whisker (the so-called
barrel column), a baseline was computed by time-averaging the images acquired in the two
seconds before stimulus onset. Each frame in the trial was divided by this baseline frame and
the “response” image was obtained by averaging the normalized images in the time interval 1
to 2 seconds post stimulus. We defined the position of the barrel in the image as the absolute
luminance minimum across the whole baseline-normalized image, following a smoothing
procedure to remove local noise fluctuations. The procedure resulted in a dark spot
(approximately 200 µm diameter) at the barrel location (Figure 2D,E).

During the same imaging procedure, we also acquired an image under green LED
illumination (527 nm) that highlighted the blood vessel pattern at the surface of the cortex and
served as a reference image for the follow-up surgery (Figure 2F).

2.6. Setting up the optical window

During a second surgical procedure that followed immediately after the intrinsic imaging
session, we removed a 4 mm diameter disk of skull over the barrel cortex while preserving
the dura, (Figure 1E) centered on the positioning of barrel C2 derived from the first intrinsic
imaging session. A 5 mm diameter glass coverslip was then glued to the sides of the
craniotomy, followed by dental cement reinforcement, thereby providing a permanent optical
window centered on the barrel cortex and allowing both the readout of intrinsic signals and
the photostimulation of Channelrhodopsin positive neurons in barrel cortex (Holtmaat et al
2009). After this step, the mouse was left to rest for up to 10 days to recover and to check that
the optical window stayed clear in the long run. Out of 18 mice that we implanted with an
optical window, 10 showed a clear window after 14 days, and remained clear until we
terminated the experiments, up to 2 months later. Mice without a clear window were removed
from the experiments.

2.7. Implantation of silicon probes over M1

A third surgery was then performed under isofurane anesthesia, this time to setup a chronic
32 channels multisite extracellular electrode (8 tetrodes across 4 shanks, Neuronexus A4x2-
tet-5mm-150-200-121-CM32) centered over the whisker motor cortex (vM1) (Ferezou et al 2007, Zagha et al. 2013). We followed an established protocol (Okun et al 2016) to insert the probe into the cortex and then attach it to the mouse skull. We made a small, approximately 1 mm² opening in the skull above the stereotactic coordinates of vM1 (A: 1.5 mm L: 0.6 mm from bregma, Figure 1E). We then resected the dura to reveal the cortex, and positioned the electrode above the opening using the stereotax arm. After a slow insertion of the electrode tip into the cortex (1 µm/s) down to layer 5 (700 µm), the craniotomy was filled with a fast set, low-toxicity silicon adhesive (Kwik-Cast, WPI) and the electrode connector was attached to the skull using dental cement primed with a thin coat of cyanoacrylate glue.

After one week of recovery, signals were recorded by plugging an extracellular recording system onto the electrode (Blackrock Cerebus). After high-pass filtering and thresholding to extract spikes, a qualitative analysis of the spiking activity was carried. Spiking activity recorded up to 2 months after electrode implantation showed no sign of degradation.

2.8. Identification of neurons with stable spiking activity

Recorded activity was manually clustered within the space of the amplitude of the spikes acquired in the 4 electrodes, using the online spike sorting software (Blackrock Microsystems Central) provided with the extracellular recording system. After this initial spike sorting, recordings were obtained on five consecutive days. A subset of the initial units that appeared stable (in terms of spike shape and mean firing rate) was selected to be part of the rest of the experiment. The pre-programmed spike sorting was carried online by the acquisition system within a few milliseconds of the spike occurrence, and was used as a template for the ongoing neuronal activity.

2.9. Generating photostimulation patterns

To control neuronal activity using photoactivation, we based our experiments on a mouse strain that expresses the light-gated ion channel Channelrhodopsin specifically in pyramidal neurons (EMX-Cre;Ai27). In these mice, photostimulation of brain tissue resulted in a spiking discharge of local pyramidal neurons (Madisen et al 2012). We took advantage of the large scale optical window to project complex spatial patterns that spanned the whole vS1 (Roy et al 2016). We designed photostimulation patterns that were carefully aligned on the barrels of vS1 (Figure 2A,B), and adhered to the discrete nature of these columnar structures by being composed of photostimulations discs (225 µm diameter, light intensity 25 mW/mm²) aligned on the barrels. To position accurately this shared pattern on the mouse barrel, we carried a new intrinsic imaging session, this time through the optical window (Figure 2D-I). We acquired the position of at least 3 barrels scattered across the barrel field. We then used these measured barrels to align at the surface of the cortex an average barrel pattern (Figure 2J) acquired in another study in adult mice of the same strain background (Knutsen et al 2016). Photostimulation discs were centered on this pattern (Figure 2K). These operations were done using a custom-made Python software.

2.10. Online control of the hardware

Access to the spike-sorted ongoing activity was provided by the extracellular recording system through a C++ API, via a UDP ethernet link. We built a Qt-based application (the “master” application) that pooled the spikes emitted by the acquisition system, and kept in memory only the spikes that were part of the user-selected units.

This master application was tasked with the control of the photostimulation system as
well as the reward system, based on the spiking activity. To ensure that performance was not affected by user interactions, the graphical user interface was implemented in a second Qt thread. It provided a readout of the ongoing spiking activity recorded in vM1; a visualization of the current photostimulation feedback, as well as a widget to adjust the parameters that affect the feedback based on the spiking activity (Video 1).

The master computer also controlled all the analog and digital inputs and outputs of the experiment. They were channeled through the same device used for electrophysiological acquisition system (Blackrock Cerebus NSP). This has the advantage of sharing the same clock with the electrophysiological data. The same system also drove the piezoelectric whisker stimulator and triggered the acquisition of frames during intrinsic imaging sessions. Finally, during behavior, it triggered the opening of the valve to deliver water rewards to the water restrained mice, and recorded each time the photoactivation pattern was updated.

2.11. Reward mechanism

In our behavioral paradigm, a range of firing rates were associated with rewards in order to promote a controlled modulation of the firing rate by the mouse. To establish this rewarding schedule, the mouse access to water supply was restricted to a single 30 min time slot at the end of each training days, and free water on the week-ends. This water schedule ensured that the mice were thirsty at the start of the behavioral trials. Water droplets were delivered through a lickport system (Estebanez et al 2017) set within reach of the head-fixed mouse tongue (Figure 3A). This device was under control of the master computer program, and lick and rewards were displayed on the graphical user interface.

2.12. Acute recordings of vS1 neurons activity during photostimulation

To ensure that the photostimulation patterns did result in an activation of neurons in vS1, we setup a control experiment to record the activity of vS1 neurons during a standard photostimulation pattern over vS1. To do, we implanted mice with a head-holder and optical window over vS1 following the standard procedure (sections 2.4 to 2.6). On the day of the planned recording, we anesthetized the mouse using isoflurane. Using a diamond-coated dental bur, we cut the glass open over a small section. We then slid an acute 32 channels extracellular electrode (Neuronexus A1x32-Poly3-5mm-25s-177-A32) through this opening, below the glass window at a 45° angle, down to approximately 300 µm depth. Finally, we applied 225 µm diameter illumination spots through the glass window, either apical to the electrode tip, or at 300 or 600 µm away from the electrode.

2.13 A Go/NOGO task to assess the detection of vS1 photostimulation

To test if vS1 photostimulations could be detected and integrated in a behavioral sequence, we designed a GO/NOGO task where a mouse was rewarded upon the correct detection of a vS1 photostimulation pattern. Trials started with an auditory cue followed by a random 4-9 s period where the animal must refrain from licking. After this period, photostimulation started (500 ms, 7 ms ON / 3 ms OFF flicker, 100 Hz). Rewards were delivered after 5 licks during the photostimulation or the following 500ms.

To ensure that the mice detected the photoactivation via the activation of neurons and vS1 and not through its visual system, we applied a flickering blue light source to the eyes of the mouse as a distractor. Finally, during the experiments we played a white noise sound background to limit the distraction from reward valve clicks.

To check that photostimulations could really not be visually detected by the mice, we included in the behavior a sham condition (20% of the trials) where the photostimulation was
applied to the dental cement next to the optical window.

3. Results

3.1. Pace and lag of the feedback loop

To assess the refresh rate of our closed-loop, we quantified the pace of the translation between spiking rate and photostimulation by monitoring the photostimulation display with a photodiode (PDA10A-EC, ThorLabs) while it was forced to flip between dark and bright frames at each program loop iteration. Except for the systematic flicker of the display, the setup was not altered for this test. The calibration was carried while a 500 Hz mean spiking rate load was applied as an input to the system. We found that the program main loop runs every 1.06 ms (mean) with a low variability (SD 0.28 ms, Figure 3B).

Beyond the refresh rate, the time needed for information to propagate from one side of the setup to its end is critical. This end to end latency of the setup (the so-called lag) can have a major impact on the loop performance. In particular, a large lag in the hundreds of milliseconds may make the information fed to the cortex entirely mismatched to the ongoing motor output. To estimate the lag, we timed the delays in the full loop using a modified feedback algorithm that flashed a bright frame as soon as a spike was read. To fully control the input during this test, we applied an artificial electrical pulse to the input stage of the electrophysiology recording system, which interpreted it as a spike. The timing of this artificial spike was used as a reference to measure the delay to update the photostimulation, as measured by a photodiode. Using this setting, we found that the loop operated with a hardware-related end to end latency of 12.3 ms (mean, SD 3.03 ms, Figure 3C) between the spiking input and frame output.

3.2. Validation of vM1 electrophysiological recordings

Next we checked that in this setting the chronic recordings obtained in vM1 were not impacted by artifacts related to the closed loop operation. In particular we looked at the potential impact of mouse licks as well as the photostimulation flicker applied on vS1.

To do so, we implanted one mouse with both a chronic electrode in vM1 and an optical window over vS1, and we trained this mouse to licks to obtain ad-libitum water rewards. Next, in this mouse we acquired with the implanted vM1 electrode both the local field potential (LFP) and the multiunit activity, while the mouse was licking and a photostimulation was applied.

Visual inspection of electrophysiological traces revealed no sign of lick (Figure 3D) or photostimulation-related artifacts (Figure 3E). Next we quantified these potential artifacts by computing the distribution of the amplitude of LFP modulations over a 10 ms window around licks and photostimulation (1000 trials), compared to the distribution of LFP amplitude in random 10 ms windows. For both photostimulations and licks, test and control amplitude distributions were not significantly different (Mann-Whitney p>0.05). Therefore, we argue that in our settings, electrophysiological recordings in vM1 were devoid of the main potential sources of artifacts.

3.3 Activation of neurons in vS1 by the photostimulation

We then ensured that the photostimulations applied to vS1 are able to activate neurons in the targeted area. To this aim we recorded extracellularly the activity of vS1 neurons in two anesthetized mice while photostimulations were applied over the optical window (see
methods, Figure 4A). We projected 225 µm diameter photostimulation spot onto vS1 following different distances from the electrode: next to the electrode recording site, 300 µm and 600 µm away. These photostimulations were flickered (5 ms ON/ 5 ms OFF) during 1.5 s each, and different positions were presented at random times. We found that photostimulation spots next to the electrode led to a fast activation of recorded putative pyramidal neurons, while the same pattern of photostimulation led to much diminished spiking activation at 300 and 600 µm away from the recorded electrode (example in Figure 4B,C, population analysis in Figure 4D,E). Overall, we found that with these settings we could trigger timely spiking in vS1 neurons, and that the spatially structured photostimulations led to spatially structured neuronal activation in vS1.

3.4 Behavioral demonstration of the detection of the vS1 photostimulations

To assess if the photostimulations can actually be perceived by the mouse and result in a behavior, we trained two mice in a GO/NOGO photostimulation detection task (see methods, Figure 5A). The mice were implanted with a headpost and a glass window. We used intrinsic imaging to locate the barrels through the glass window, and we generated photostimulation of individual barrels that formed a bar-like stimulus, including 5 barrels along the C2 row (a photostimulation similar to the pattern shown in Figure 6B). We trained the mouse to detect this stimulus in order to obtain a water reward (Figure 5A, left).

Following training (approximately 10 days, twice daily), the mice learned to lick specifically after the trial photostimulation. This was not the case in sham trials where the photostimulation was applied on the dental cement next to the optical window (Figure 5B,C). Therefore, we conclude that the vS1 photostimulation-based feedback could be sensed by the mice and that this detection occurred through the optogenetic activation of vS1 neurons.

3.5 Mice are able to perform a behavioral task using the closed-loop

To test the full closed-loop of the BMI and demonstrate its capabilities, we implemented a task that relied on the artificial vS1 inputs and vM1 outputs. This task was based on the control of a virtual bar that deflected virtual whiskers during its rostrocaudal displacement (Figure 6A). Contact of the virtual bar with the virtual whiskers led to photostimulations of the corresponding barrels using a simple one to one correspondence between specific firing rate ranges and a given pattern of photostimulation (Figure 6B,C and Video 1).

The 1D position of the virtual bar was directly tied to the pooled activity of the units that were recorded in vM1, after binning with a sliding 0.1 s box kernel to improve the smoothness of the control of the bar. Low firing rate corresponded to a caudal position of the bar, and high firing rate to a rostral position.

The photostimulation patterns were derived from the 24-whisker pattern of photostimulation (Figure 2K) by selecting subsets of the photostimulation discs that mimic a bar crossing the whiskerpad (Figure 6B). Overall, large swings in population firing rate resulted in an almost simultaneous back and forth motion of a bar-like photostimulation in vS1 (Video 1 and Figure 6C, obtained in a non-trained mouse). These stimulus patterns are known to drive strongly barrel cortex responses (Jacob et al 2008, Drew et al 2007). To minimize Channelrhodopsin desensitization that results from permanent photoactivation (Nagel et al 2003), the patterns were pulsed at 100 Hz (50% duty cycle) by using a built-in flickering capability of the projector.

The goal of this task was to train the mouse to position the bar within a limited range of firing rate defined by the experimenter. To this aim, the mouse was water restricted to transform water droplets into valuable rewards. The mouse could obtain such water drops by
licking a water port at the specific time when it managed to hold its firing rate within the target range. To limit opportunistic rewards only the first lick was rewarded after the firing rate entered the rewarded zone.

To test this behavior, we implanted two mice with an optical window over vS1 and a chronic extracellular electrode in vM1. After recovery, the mouse was head-fixed. The photostimulation was aligned over the barrels in vS1 using a custom made calibration software driving the camera and the projector (Figure 2).

To train the water-restricted mouse to perform the task, we shaped it with the following steps. On the first training session we let the mouse lick freely to obtain water. Then on the next sessions, we conditioned the water delivery on population firing rate that was inside the target firing rate range. Initially, this range was close to the baseline population firing rate, and it was crossed frequently at random. But over the course of the trials, we moved the rewarded firing rate range away from the baseline firing rate. Within one to two weeks of training to the task once a day for 40 minutes, we found that the mouse had learned to (1) perform large swings of firing rate to attain the rewarded range, and (2) lick timely and therefore obtain water rewards (example in Figure 6D, left and Figure 6E). To assess the impact of the photostimulation on this behavior, we then tested the exact same behavior, but this time with the photostimulation feedback turned off. We found that in this condition, there were still fast increases of firing rate, but they were not anymore simultaneous to licks and led to very few rewards in both mice (Figure 6D,E).

Overall, we showed that mice can learn to solve a behavioral task in the context of our closed-loop BMI, and that the behavior performance relies on the artificial feedback provided in vS1.

4. Discussion

4.1. Choice of the vS1/vM1 implantation

The decision to develop a feedback system that targets directly the cortex and not the periphery ensures that, in the context of a medical treatment, it could operate even in patients with a number of peripheral disabilities. Similarly, invasive cortical readout interfaces are designed to bypass a defective spinal cord (Collinger et al 2013, Hochberg et al 2006, Hochberg et al 2012, Ajiboye et al 2017). This invasive feedback strategy has been recently selected to setup somatosensory feedback interfaces (Tabot et al 2013, Flesher et al 2016), as well as to replace other senses such as vision (Dobelle et al 1974, Dobelle et al 2000).

Within the cortex, the choice of vS1 and vM1 among the many possible input/output areas is motivated by three reasons. First, S1 and M1 are nearby areas – in particular in humans. Therefore, in the perspective of a medical application, both inputs and outputs could be grouped into a compact “neural port” attached to the skull in a stable way. Beyond this practical reason, we selected S1 for feedback as this area is specialized in the processing of touch input (Estebanez et al 2018), but also proprioceptive inputs (Kim et al 2015b), which are the two main inputs that should be conveyed from an artificial prosthesis. Third and equally important to our focus on the vM1/vS1, there is a large bundle of axonal fibers that projects from vS1 to vM1 (Ferezou et al 2007, Welker et al 1988, Zakiewicz et al 2014). This suggests that inputs to vS1, including artificial inputs, may be quickly and reliably relayed to vM1 for integration into motor commands, as confirmed by functional studies in vM1 (Zagha et al 2015, Fassihi et al 2017, Ferezou et al 2007).

Finally, we should mention that this short latency connection between vS1 and vM1...
does not preclude the projection of vS1 inputs to other brain areas, as well as the control of vM1 activity from other brain areas (Figure 3A). Therefore, this choice does not restrict the involvement of the whole brain in solving tasks that are interfaced through the closed-loop BMI. Instead, it only ensures that a high-performance, reflex pathway is available to carry part of the behavior.

4.2. Optogenetic control of vS1 activity via a DLP projector

We chose to implement this BMI feedback using the photostimulation of channelrhodopsin-expressing pyramidal neurons in vS1, while so far attempts at including a feedback in a BMI have always relied on electrical microstimulation of S1. We were interested in taking advantage of the high level of control on the spatial organization that is provided by optogenetics in the mouse. In addition, this technique makes it possible to restrict the neuronal activation to a specific cell subtype (Cardin et al 2010). Finally, photoactivation of Channelrhodopsin does not disturb the electrophysiological recording in vM1, in contrast with electrical microstimulations (O’Doherty et al 2011).

Using this versatile feedback device, we will be able to explore a large array of feedback schemes, including feedback schemes that adhere to the somatotopical map like the one we demonstrate here, but also photostimulation patterns that are independent from the functional maps. Both high frequency, up to kHz modulations, and slower gray scale displays may be also explored. This approach was inspired by a number of studies that showed that photoactivation of Channelrhodopsin expressing neurons was a promising way to substitute failing physiological sensory inputs at the periphery. In particular, in the visual pathway, peripheral photostimulations of the retina can be substituted to physiological light activation, including complex coding properties of retina neurons (Greenberg et al 2011). These studies aim to develop a spatially structured (Reutsky et al 2007), multichannel, visual-like input to the central nervous system. Although we aim to achieve a comparable sensory substitution, we are aiming at the cortex, and this led us to different choices. In particular, the photostimulation characteristics are profoundly different when targeting vS1 versus the retina, which is a high-resolution and spatially extended imaging layer with well known microcircuitry, in sharp contrast with the lower spatial resolution, higher temporal resolution and poorly know microcircuits of vS1. Overall, beyond the shared use of optogenetics, we believe that cortical optogenetic substitution at cortical level require a whole different set of development compared to its use at the periphery, for instance at retina level.

4.3. Readout of vM1 activity

To readout the activity of the subject, we have chosen to use extracellular electrodes implanted in the primary motor cortex. This ensures a stable access to the activity of neurons (Okun et al 2016). The use of extracellular invasive recordings to ensure the data readout is a proven technique (Arduin et al 2013, Arduin et al 2014) to drive prosthetic devices, including forearm/hand surrogates with a large number of independent actuators (Collinger et al 2013, Hochberg et al 2012, Ajiboye et al 2017) which would highly benefit from a rich feedback. However, the choice of extracellular recordings also has its downsides. In particular, in contrast to optical methods, it cannot capture the spatial organization of the functional activity in M1, despite the functional maps that structure this area, in particular corresponding to the body plan (Penfield et al 1937). In addition, in the long run, electrodes tend to damage the structure where they have been inserted, by causing tissue inflammation (Karumbaiah et al 2013). Still, month long recordings with chronic silicon probes are possible (Vetter et al 2004), in particular with the tethered connector and thin (15µm thick) silicon probe design.
that we selected (Karumbaiah et al 2013). An alternative design choice would be to extend the optical window to reveal M1, and use optical methods to measure the tissue activity, including genetically encoded calcium indicators such as GCaMP6 (Chen et al 2013). Such an all-optical BMI has already been attempted recently using two photon microscopy to readout activity-related fluorescence fluctuations in individual M1 neurons (Prsa et al 2017), but alternate strategies could also be used to record the activity at the scale at which the photostimulations have been applied, potentially by using the camera that captured the intrinsic imaging signal. By this means, we could acquire meso-scale fluorescence signals in mice that express a calcium indicator in M1 (Minderer et al 2012).

4.4 End-to-end latency of the closed loop

Due to the low latency of inputs from the periphery to vS1 (LeCam et al 2011) as well as the potentially disruptive impact of lags on closed-loop settings (Kim et al 2005) we focused our attention on a technological solution that would result in a low-latency transmission of information. To do so, we chose to perform all our programming in a high-performance language (C++) and we relied on a low lag electrophysiology acquisition system and photostimulation device.

Still, there are naturally occurring lags in all sensorimotor loops, including the whisker system, and these lags might be worth mimicking in an artificial connection between S1 and M1 to comply with what M1/S1 neurons may be expecting during natural behavior.

Several studies have timed the whisker system sensorimotor loop in the awake mouse, and found that early responses in vS1 occur within 7 ms of the whisker stimulus onset. The transmission of this input from vS1 to vM1 requires approximately 8 ms (Ferezou et al 2007). Finally, electrical and optogenetic microstimulation experiments showed that M1 activation triggered whisker movements after at least a 20 ms delay (Matyas et al 2010, Auffret et al 2017). Therefore, in total, the natural whisker system full sensorimotor loop from vM1 motor command to vS1 feedback takes up at least 27 ms, a delay larger than the 12.3 ms we measured in our artificial BMI loop. This short latency gives us the opportunity to play with the lag in our BMI loop, and decide if it should precede, match or lag behind the physiological end to end latency of the sensorimotor loop.

In addition to these hardware latencies, one should note that our artificial loop is also impacted by delays related to the time window that is required to translate instantaneous firing recorded in vM1 into a motor command. At the moment, we have chosen to rely on a very simple algorithm, namely a 100 ms binning kernel which translates raw spikes into a smooth firing rate that constitutes the motor command. In further developments, several strategies could be developed to reduce the impact of this integration window. One strategy is to use more elaborate integration algorithms. But beyond processing strategies, probably the most efficient way to reduce the need for a long integration window is to increase the average firing rate recorded in vM1. One way to achieve this goal is by training the mouse to shift the activity of the vM1 neurons to higher firing rate ranges. Another option would be to merge a larger number of neurons into the population firing rate. These additional neurons could be acquired by increasing the number of independent electrode recording sites by moving from a 32 to a 64 channel electrode.

5. Conclusion

We have reported on the development of an invasive BMI that includes a versatile
optogenetics-based feedback system capable to produce arbitrary spatio-temporal patterns of stimulation onto the surface of the cortex, at a mesoscopic scale (150 µm patterns). In the head-fixed mouse, we used intrinsic imaging to position an optical window above vS1. Using the same optical window, we applied spatio-temporally structured photostimulation patterns apically on the cortex, which aligned on the barrels. In the same mice, we implanted an extracellular silicon multielectrode in vM1. And we glued together these components using C++ programs. Finally, we benchmarked the full BMI loop and showed that the photoactivation of vS1 led to neuronal spiking that could be detected by the mouse and support a BMI task.

Overall, this setup has the potential to be a versatile platform to explore different somatosensory feedback strategies in BMIs, and more generally to evaluate the attractiveness of the cortical artificial feedback strategy.

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Figure 1. A BMI design including a mesoscopic sensory feedback in vS1. (A) Overall principle of the BMI. Red path: the readout of vM1 spikes. Blue path: Feed-back synthesis and projection in vS1 of an Ai27 x EMX-Cre mouse. Channelrhodopsin is expressed in pyramidal neurons, which are therefore light-triggered. (B) Optical path of the macroscope used for both vS1 patterned photoactivation and intrinsic imaging. (C) Example photostimulation pattern projected by the macroscope on a flat, glossy paper target. (D) Distribution of photostimulation intensity across the discs shown in C. (E) Configuration in stereotactic coordinates of vS1 optical window and vM1 electrode implant.
Figure 2. Functional identification of vS1 anatomy via intrinsic imaging.
(A) Spatial organization of the whiskers on the mouse face. Red: Straddlers. Green: C2 whisker. (B) Spatial organization of the barrels in layer 4 of the barrel cortex, in the context of the mouse S1 somatotopy (gray background). Arrows point to the rostral and lateral stereotactic axes. (C) Intrinsic imaging setup. A whisker stimulation piezo-electric bender moves a selected whisker of an anesthetized, head-fixed mouse, under the macroscope used to convey red light reflected by the cortex to the CCD 12 bits camera. (D) Time course of the intrinsic signal through the skull, obtained by averaging the signal in the dark area in E. Blue: whisker stimulation time. Zoom-in shows signal time course. Baseline (purple) and response (orange) time windows are also shown. (E) Intrinsic imaging response map. Cyan arrows: alignment marks also shown at the same location in F,H and I. (F) Reference image of the surface blood vessels obtained with green illumination. (G-I) Same as D-F, this time through the optical window. (J) Putative location of barrels at the surface of vS1. Dashed line: localization of the intrinsic signal (50% of peak response). Points: shared map of barrels location (see main text). Green: D4 whisker. Same for A2 (blue) and C1 barrel (red). (K) Full photostimulation pattern aligned to the intrinsic imaging localized barrels.
Figure 3. Short-latency translation from spiking rate to photostimulation. (A) Summary of the information flow through the setup. Left: extracellular recordings are spike-sorted and resulting spikes are fed to the master computer. Right: the master computer acquires lick times, controls the water reward of the mouse, and updates vS1 photostimulation. (B) Distribution of the loop pace. Black line: mean 1.06 ms, SD 0.28 ms. (C) Distribution of the end to end latency of the setup, measured from spiking input to change of the photostimulation. Black line: mean 12.3 ms, SD median 3.03 ms. (D) Case study showing the lack of artifactual modulation of the local field potential (LFP) and the multiunit firing rate recorded in vM1 following mouse licks. (E) Same as D for potential photostimulation-related artifacts.
Figure 4. Photostimulation of vS1 results in a strong, local increase of vS1 neurons firing rates. (A) Neuronal activity in the vS1 area of anesthetized mice is acquired using an acute electrode slid below the glass window. Photostimulation spots are applied next to the electrode; 300 µm and 600 µm away. (B) Case study. Mean and SEM (gray background) of the spike shape (top) and autocorrelogram (bottom) of a selected single unit. (C) Photostimulation-aligned peristimulus time histogram of the selected unit for illuminations located at electrode location (0 µm), 300 or 600 µm away from electrode. Blue background: illumination (5 ms ON, 5 ms OFF flicker). (D) Population (2 mice, 18 neurons) mean and SEM (gray background) of the neuronal activity aligned on the photostimulation next to the electrode. Blue background: illumination. (E) Box plots showing the population analysis of the firing rate measured at baseline, and in the 1 s following photostimulation onset.
Figure 5. Photostimulation of vS1 are detected by the mice and integrated in a behavioral task. (A) Awake mice receive photostimulations either in vS1 through the optical window, or on the dental cement of their head cap (sham). (B) Licking pattern in a trained mouse. Top: licking peristimulus time histogram on the photostimulation (blue background), black line for vS1 photostimulation and red line for sham trials. Bottom: raster plot of licking across all trials. Same color code as in top panel. (C) Proportion of trials where the mouse licked in a timely manner, in vS1 photostimulation trials (black) versus shams (red).
Figure 6. Proof of concept of a simple behavior using the closed-loop BMI. (A) Using the readout from vM1 neurons, the mouse must learn to move the position of a virtual bar (red). This bar is located inside a virtual whiskerpad, and when it touches the virtual whiskers, this results in a photoactivation of the corresponding barrels in vS1. (B) Frames projected onto vS1, as a function of the level of vM1 firing rate from low (frame 0) to high (frame 7). White discs: photostimulated barrels. Grayed discs: non-photostimulated barrels. (C) Example of population vM1 activity in an awake non-trained mouse. Raster of activity and population firing rate (bottom) and the resulting vS1 photostimulation frame identity across time (top). The translation of firing rate to projection happens within upper and lower firing rate limits (dashed lines). Rewards are dispensed when firing rate is maintained within a narrow range (frames 3 and 4, green). (D) Example of the behavior of a mouse that learned the task, including licks (bottom) and the population firing rate that controls the virtual bar (middle). Green interval: rewarded. Left: optogenetic feedback activated. Right: feedback deactivated leads to a drop in reward rate. (E) Rewarded licks rate (Hz) in the two mice that were trained to the behavior, with versus without feedback.