

# Hypothalamic Control of Forelimb Motor Adaptation

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The ability to perform skilled arm movements is central to everyday life, as limb impairments in common neurologic disorders such as stroke demonstrate. Skilled arm movements require adaptation of motor commands based on discrepancies between desired and actual movements, called sensory errors. Studies in humans show that this involves predictive and reactive movement adaptations to the errors, and also requires a general motivation to move. How these distinct aspects map onto defined neural signals remains unclear, because of a shortage of equivalent studies in experimental animal models that permit neural-level insights. Therefore, we adapted robotic technology used in human studies to mice, enabling insights into the neural underpinnings of motivational, reactive, and predictive aspects of motor adaptation. Here, we show that forelimb motor adaptation is regulated by neurons previously implicated in motivation and arousal, but not in forelimb motor control: the hypothalamic orexin/hypocretin neurons (HONs). By studying goal-oriented mouse-robot interactions in male mice, we found distinct HON signals occur during forelimb movements and motor adaptation. Temporally-delimited optosilencing of these movement-associated HON signals impaired sensory error-based motor adaptation. Unexpectedly, optosilencing affected neither task reward or execution rates, nor motor performance in tasks that did not require adaptation, indicating that the temporally-defined HON signals studied here were distinct from signals governing general task engagement or sensorimotor control. Collectively, these results reveal a hypothalamic neural substrate regulating forelimb motor adaptation.

**Key words:** Key words: hypothalamus; motor adaptation; motor learning; movement; orexin/hypocretin; upper limb

## Significance Statement

The ability to perform skilled, adaptable movements is a fundamental part of daily life, and is impaired in common neurologic diseases such as stroke. Maintaining motor adaptation is thus of great interest, but the necessary brain components remain incompletely identified. We found that impaired motor adaptation results from disruption of cells not previously implicated in this pathology: hypothalamic orexin/hypocretin neurons (HONs). We show that temporally confined HON signals are associated with skilled movements. Without these newly-identified signals, a resistance to movement that is normally rapidly overcome leads to prolonged movement impairment. These results identify natural brain signals that enable rapid and effective motor adaptation.

## Introduction

Regulation of skilled arm movements has been a subject of intense research (Franklin and Wolpert, 2011). In human experiments, robotic manipulanda produce controlled and reproducible velocity-dependent force field perturbations, that allow fundamental elements of sensorimotor control to be dissected

(Shadmehr and Mussa-Ivaldi, 1994; Wolpert and Miall, 1996; Shadmehr et al., 2010; Franklin and Wolpert, 2011). These elements include the evaluation of sensory expectations based on motor commands and internal models of sensorimotor transformations that determine motor outputs (Kawato, 1999; Thoroughman and Shadmehr, 2000; Shadmehr et al., 2010; Wolpert et al., 2011; Wolpert and Flanagan, 2016). Internal models can be seen as libraries of motor commands. They implement a model of limb and task dynamics which transforms a desired movement trajectory into motor commands (Wolpert and Kawato, 1998). In unfamiliar motor tasks, the internal model uses the discrepancy between the desired and actual movement trajectory (“sensory prediction error”) to iteratively update motor commands across attempts and thus reduce error (“sensory error-based motor adaptation”; Wolpert et al., 1998, 2011; Kawato, 1999; Thoroughman and Shadmehr, 2000; Shadmehr et al., 2010; Wolpert and Flanagan, 2016).

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Alternatively, when a reward is given after each successful attempt, adaptation can also occur according to changes in this feedback (“reward-based motor adaptation”; Izawa and Shadmehr, 2011). The relative contributions of sensory versus reward-based learning to motor adaptation depend on the experimental paradigm, and on whether motor adaptation is primarily guided by sensory errors rather than reward rate (Mazzoni and Krakauer, 2006; Schaefer et al., 2012; Mathis et al., 2017).

Error-based motor adaptation is impaired in common disorders, e.g., after strokes (Takahashi and Reinkensmeyer, 2003; Smith and Shadmehr, 2005; Krakauer, 2006; Scheidt and Stoeckmann, 2007; Butcher et al., 2017). While the behavioral consequences of lesions in certain brain regions have been well characterized, we know little about how genetically-defined neurons influence specific elements within the frameworks of error-based motor control. In human studies, this knowledge is limited by the inability to record and manipulate genetically-defined neurons. In rodent models, it has been limited by a lack of robot-assisted paradigms that provide reproducible perturbations. In particular, no studies have been performed using velocity-dependent force fields, which enable dynamic remapping of internal models and quantification of neural data in the context of adaptation frameworks (Butler et al., 2000; Gandolfo et al., 2000; Li et al., 2001; Franklin and Wolpert, 2011; Mathis et al., 2017).

Among the multiple central nervous system modules implicated in adaptive motor control, the cortex, basal ganglia, cerebellum, brainstem, and spinal cord have been intensively studied (Bear et al., 2015; Arber and Costa, 2018). In contrast, hypothalamic orexin/hypocretin neurons (HONs; de Lecea et al., 1998; Sakurai et al., 1998) have received much less attention. Although HONs were originally studied in the context of arousal and motivation (Mahler et al., 2014; Sakurai, 2014), abundant evidence implicates them in central motor control (Hu et al., 2015). HONs directly innervate and activate the classic motor systems, including the motor and somatosensory cortices, the basal ganglia, the cerebellum, and the spinal cord (Peyron et al., 1998; Cutler et al., 1999; Nambu et al., 1999; van den Pol, 1999; Date et al., 2000; Korotkova et al., 2002; Bayer et al., 2004; Yamuy et al., 2004; Biswabharati et al., 2018). At the functional level, however, HONs have only been studied in relation to innate locomotion and vestibular control (Chemelli et al., 1999; Kiyashchenko et al., 2001; Zhang et al., 2011; Feng et al., 2020; Karnani et al., 2020), and the roles of HONs in acquired, refined motor skills remain undefined.

Here, we applied robotics inspired by classic human upper limb studies to a mouse model for studying HON activity. During a skilled forelimb task, we exposed mice to velocity-dependent force fields designed to require dynamic motor adaptation, measuring analogs of behavioral elements typically analyzed in human studies (Schwarz et al., 2019; Kanzler et al., 2020). Recording and control of HONs in this context allowed examination of their involvement in skilled motor performance.

## Materials and Methods

### Genetic targeting of optical sensors and actuators of neural activity

Animal experiments were performed in accordance with the Animal Welfare Ordinance (TSchV 455.1) of the Swiss Federal Food Safety and Veterinary Office, and approved by the Zurich Cantonal Veterinary Office. Subjects were adult male C57BL/6 mice over the age of seven weeks. Mice were housed in a reversed 12/12 h light/dark cycle. Experiments were performed during the dark phase. The specific targeting of GCaMP6s and ArchT to orexin neurons was performed using the

same surgical and genetic tools described and histologically validated in our previous study (Karnani et al., 2020). Briefly, to target the optogenetic inhibitory actuator ArchT to orexin neurons, we injected AAV1-hORX.ArchT.TdTomato (1:20 dilution, titer:  $1.03 \times 10^{13}$  GC/ml; prepared by Vigene Biosciences) into the lateral hypothalamus. To target GCaMP6s to orexin neurons, we injected AAV1-hORX.GCaMP6s (titer:  $2.0 \times 10^{13}$  GC/ml; prepared by Penn Vector Core) into the lateral hypothalamus. To control for possible heating artifacts because of laser manipulation, a subset of mice were implanted with optic fibers, with no actuators expressed. For stereotaxic brain injections, mice were anesthetized with isoflurane (1% at 1 l/min), injected with meloxicam (10  $\mu$ l/g of body weight, s.c.) for analgesia, and placed into a stereotaxic frame (Kopf Instruments). A craniotomy was performed, and a glass pipette mounted on a Nanoject II injector (Drummond SCI) was used to inject the AAV vectors into the hypothalamus; 400 nl of ArchT AAV vector or 150 nl of GCaMP AAV vector was injected per hemisphere at the following coordinates: bregma  $-1.55$  mm, midline  $\pm 1$  mm, depth 5.4 mm skull surface (González et al., 2016a; Kosse and Burdakov, 2019). Mice recovered from surgery for at least 10 d before experiments.

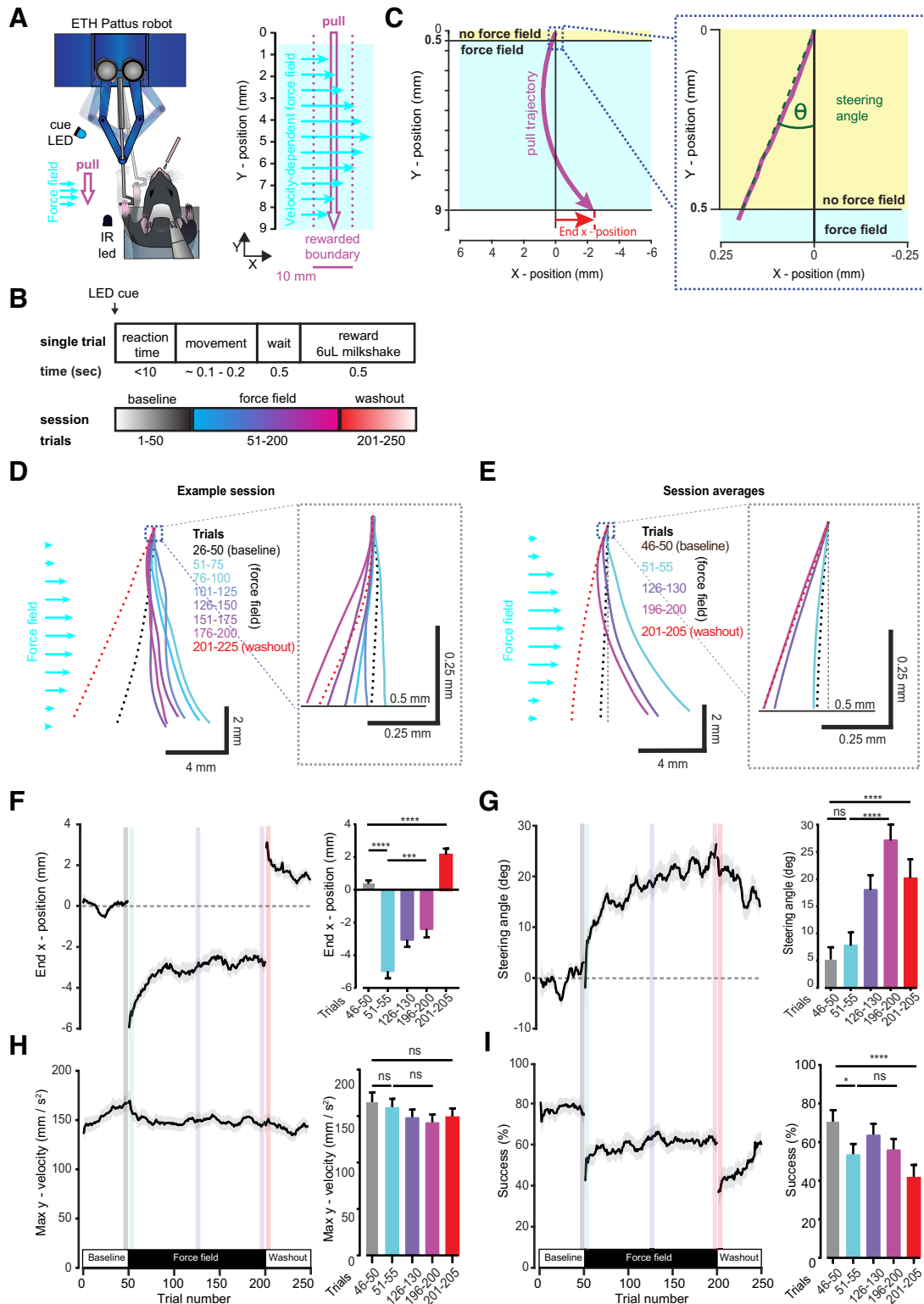
### Mouse-robot interaction

Mice used their left forelimb to interact with a robotic arm to obtain rewards. The robot was based on the ETH Pattus (Vigaru et al., 2011, 2013; Lambercy et al., 2015), and re-developed to enable forelimb-motor-adaptation analysis in mice (Fig. 1A). It comprises a two-degrees-of-freedom robotic manipulandum operated by electromagnetic motors coupled with high-resolution rotary encoders (Vigaru et al., 2011, 2013; Lambercy et al., 2015). Mice were trained to interact with the robot via a horizontally oriented joystick handle fixed at the tip of the robotic arm, 5 mm in width and 1 mm in diameter. The handle could be manipulated to perform a specific motor task [in this study, to pull 9 mm in the y-dimension from starting point with lateral (x) deviations not exceeding  $\pm 5$  mm; Fig. 1A,B]. Upon successful task execution, the robot automatically delivered a milkshake reward (see behavior training, below). A blue LED cue was placed near the mouse to signify the trial state. For each trial, the handle was unlocked and a cue (blue LED turning off) informed the mouse that it could begin to pull. After the start cue, pulls were performed only in infra-red (IR) light, so sensory feedback was limited to somatosensation (Fig. 1A). The dynamic and kinematic components of the planar movements in the x-y direction, and their responses to precisely-defined force fields, were extracted in an automatically controlled and reproducible manner. During movements, the robot continuously recorded handle position and velocity at a sampling frequency of 1 kHz. During force field trials, the robot perturbed this movement by generating a force field that pushed the handle from left to right in the x-direction in a y-velocity-dependent manner (Fig. 1B). The y-velocity was linearly converted to x-direction force as described previously (Vigaru et al., 2013), producing velocity-dependent force fields similar to those used to study forelimb adaptation in humans (Shadmehr and Mussa-Ivaldi, 1994). The gain of the linear velocity to force conversion was adjusted empirically and kept constant throughout the study. Kinematic and dynamic data of the robot were recorded in LabView and transferred to MATLAB for analysis.

### Optical recording and control of neural signals

Fiberoptic implants (200  $\mu$ m in diameter, 0.39 NA, Thorlabs) were placed stereotaxically with the fiber tip above the lateral hypothalamus (bregma  $-1.55$  mm, midline  $\pm 1$  mm, depth 5.1 mm from skull surface), and attached to the skull with dental cement (Karnani et al., 2020). A custom printed head plate (Protolabs Inc) was fitted for head fixation to ensure the head was placed at a natural angle (basi-occipital plane  $\sim 22^\circ$  from horizontal; Vidal et al., 2004).

To record the activity of GCaMP6s-expressing HONs, fiber photometry was conducted using the lock-in mode of a Doric fiber photometry system (405- and 465-nm excitation, modulated at 334 and 471 Hz, respectively, with an excitation light power of  $\approx 100$   $\mu$ W at the fiber tip). Demodulated signals and TTL outputs from the ETH Pattus robot were then aligned for subsequent analysis. GCaMP6s fluorescence emitted in response to 405-nm excitation gave a real-time control for movement



**Figure 1.** Mouse-robot interactions reveal forelimb motor adaptations to defined force fields. **A**, Left, Diagram of the ETH Pattus robotic manipulandum with top-down view of a head-fixed mouse, pulling the robot handle. Right, Diagram of the skilled motor task. Mice had to pull 9 mm in the y-direction within a  $\pm 5$  mm x “tunnel” to obtain a reward. On trials 51–200, a velocity-dependent force (cyan arrows) pushed the animal’s forelimb in the x direction. **B**, Top row, Diagram of the sequence of a single trial. Bottom row, Diagram of a single force field session. The mice performed 50 trials with no force field to get a baseline (black bar), then 150 trials of force field exposure (cyan to magenta bar), followed by 50 trials of no force field to determine the washout (red bar). **C**, Graphical explanation of end x-position and steering angle. **D**, Left panel, Typical example of trajectory evolution across a force field session. The force field was present in trials 51–200, individual lines represent average of 25 blocks. Right panel, Magnification of the first 0.5 mm of the left trajectories, demonstrating preemptive steering adaptation. **E**, Averages of five trajectories taken from preforce field (black), force field (blue, purple, magenta), and postforce field (washout, red) trial blocks. Data from 33 sessions from 12 mice. **F**, Left panel, Lateral displacement at the end of pulls (end x-position), expressed as mean  $\pm$  SEM, versus trials ( $n = 33$  sessions from 12 mice). The shared x-axis for panels **F**, **H** is given in panel **H**. Right panel, End x-position within indicated trials (mean  $\pm$  SEM). RM ANOVA  $F_{(4,128)} = 86.28$ ,  $p < 0.0001$ ; Sidak’s multiple comparison tests \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ . **G**, Left panel, Same as in **F**, left panel, for predictive steering angle. Right panel, Same as in **F**, right panel, for predictive steering angle. RM ANOVA  $F_{(4,128)} = 18.07$ ,  $p < 0.0001$ ; Sidak’s multiple comparison tests \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ .

artefacts (Kim et al., 2016). For each recording session, a convex hull function was fitted to the raw 405 nm-excited signal and raw 465 nm-excited signal, then each were divided by this fit to produce a normalized  $\Delta F/F$ . The normalized 405 nm-excited signal was then subtracted from the normalized 465 nm-excited signal, then z-scored across the session. Before combining signals from different sessions (Fig. 2C,D), the mean z-scored value of  $-2$  to  $-1$  s before pull onset was subtracted from the signal to produce a “ $\Delta z$  score” value. In Figure 2E,F, “ $\Delta z$  score” values, representing the change in pull-associated signal amplitude after trial 50 (i.e., when force field was introduced in some sessions), were calculated from signal z scores as follows: (1) pull-associated signal amplitude was calculated by subtracting the lowest signal between cue and pull onset from the signal at end of pull, before the reward was given; (2) the mean value of pull-associated signals during the baseline trials 1–50 was subtracted from each pull-associated signal amplitude.

To control the activity of opsin-expressing HONs, green lasers (532 nm, LaserGlow) were connected to the bilateral fiber implants to yield  $\sim 10$ -mW light power output at each fiber tip. Since photometry recordings showed that pull-associated endogenous orexin signals started to activate between the cue and pull onset (Fig. 2C,D), laser illumination was started at the cue and terminated at pull offset. Importantly, this enabled selective temporal targeting of premotor and pull-associated orexin cell activity, without targeting orexin signals associated with reward delivery status after the pull offset, or orexin signals between trials.

### Behavioral training

After recovery from surgery, mice were food restricted to bring them to 90% bodyweight. For the first 2 d of training, they were habituated to handling and head-fixation on the robotic apparatus for around 15 min. The head plate was held by custom-built holders that positioned the mouse in the same place on a stage relative to the robotic handle across days. A Plexiglas tube that loosely encased the mouse's body also helped to keep body position similar across mice and sessions, yet allowed the mouse to fully extend its forelimb without hindrance. A small metal bolt was attached to the right side of the stage, which the mouse could hold on to with its right forepaw for stability while interacting with the robot.

In the habituation phase, the robot handle was positioned 1 mm from the stage (at the natural resting position of the mouse's left forepaw). A reward spout, gated by a TTL-operated solenoid valve (NR research), was positioned  $\sim 2$  mm from the mouth. Throughout all experiments, the spout delivered 6  $\mu$ l of milkshake 500 ms after the animal made a correct forelimb movement. Reward delivery lasted 500 ms and was accompanied by an auditory tone of the same duration. Over the first 2 d, rewards were delivered automatically any time the mouse moved the robot handle  $>0.2$  mm and for a duration longer than 40 ms. If necessary, rewards were delivered manually by the experimenter using a switch. During this phase, mice rapidly learnt to associate touching the handle with reward, typically performing 50–100 touches per day.

The next phase taught the mice to wait for a cue, and to reach for the robot handle. First, the robot handle was retracted to 2 mm from the left forepaw, and locked in place by the motors until the encoders detected no movement above 0.2 mm for 2 s. After this, the handle was unlocked and a cue (blue LED turning off) informed the mouse that a trial could be started. During this period, any time the mouse touched or moved the handle that resulted in displacement of  $>0.2$  mm for  $>40$  ms resulted in a reward. After a successful touch, the cue LED was turned back on to prompt the mouse not to move the handle. The robot then moved the handle back to the start position and locked it until the next trial. If the mouse did not initiate a trial 10 s after the cue, the handle was

again locked in place and the above sequence restarted. After 10 rewards had been delivered, the handle was then moved away from the mouse by 1 mm, and again any movement of the handle after the cue resulted in reward delivery. This step was repeated until the handle reached the final position of 15 mm from the stage. These sessions lasted up to 50 min and this training phase typically took 2–3 d.

Mice were next taught to pull the handle 3 mm in the y-dimension. Pulls shorter than 3 mm in the y-dimension, that moved outside the  $\pm 10$  mm in the x-dimension, or stopped movement of  $>0.2$  mm for  $>50$  ms, resulted in no reward and the robot being reset to the start position. Once mice successfully pulled  $\geq 6$  out of 10 consecutive trials, the y length of the pull was increased by 3 mm. After a further 6/10 successful trials, the y length of the pull was increased a further 3 mm, to the final pull distance of 9 mm. This phase typically took 3–4 d to learn.

In the next phase, the reward “tunnel” was narrowed to  $\pm 5$  mm from the x-midline. Mice had to perform above 70% successful pulls per day for two consecutive days, which typically took two to three weeks. All sessions mentioned above were terminated after 50 min, and typically allowed the mouse to perform 200–400 trials.

Sessions with force field trials were then introduced, in which a velocity-dependent force field was generated by the robot motors 0.5 mm to 9 mm along the y-dimension of the pull trajectory. Mice performed 50 preforce field trials during which they had to perform above 65% success, with the pull criteria mentioned above, else trials continued without force field until mice reached 250 trials or 50 min in the apparatus. Data from these days was excluded from further analyses because of low baseline success rate. In sessions where mice reached above 65% in the 50 preforce field (baseline) trials, the force field was then imposed until animals had completed an additional 150 pulls. After this, a further 50 “washout” trials were performed without the force field. Mice typically performed all 250 trials within 35 min. Force field and nonforce field days were randomly interleaved, avoiding session order artefacts caused by mice predicting force field onset, as well as serving as an additional control for baseline motor execution. Optosilenced and light control mice performed 3 d of control and optosilencing sessions in the force field, randomly interleaved, along with interleaving nonforcefield days. Data were discarded if it did not meet the steering angle criteria (see below, Steering angle).

### Task performance metrics

#### Pull kinematics and dynamics

Pull velocity signals were low-pass filtered using a second-order Butterworth filter with a cutoff frequency of 30 Hz. Position and velocity traces were resampled to 201 points using b-spline interpolation, to obtain a constant number of samples per trial and allow for calculation of mean trajectories and mean velocity profiles over an entire session. Trajectory plots (Figs. 1D,E, 3C) were calculated by taking the average of the x and y coordinates of the 201 interpolated points within 10 trial blocks, and then averaged across sessions. The adaptation analyses were focused on velocity and displacement metrics that are typically extracted in human studies (Schwarz et al., 2019; Kanzler et al., 2020).

#### Max y-velocity

The velocity metric (max y-velocity) was measured at the peak of the y-component of velocity for each pull.

#### Lateral displacement end x-position

The lateral displacement (end x-position) was measured at the final sample of x in the trajectory.

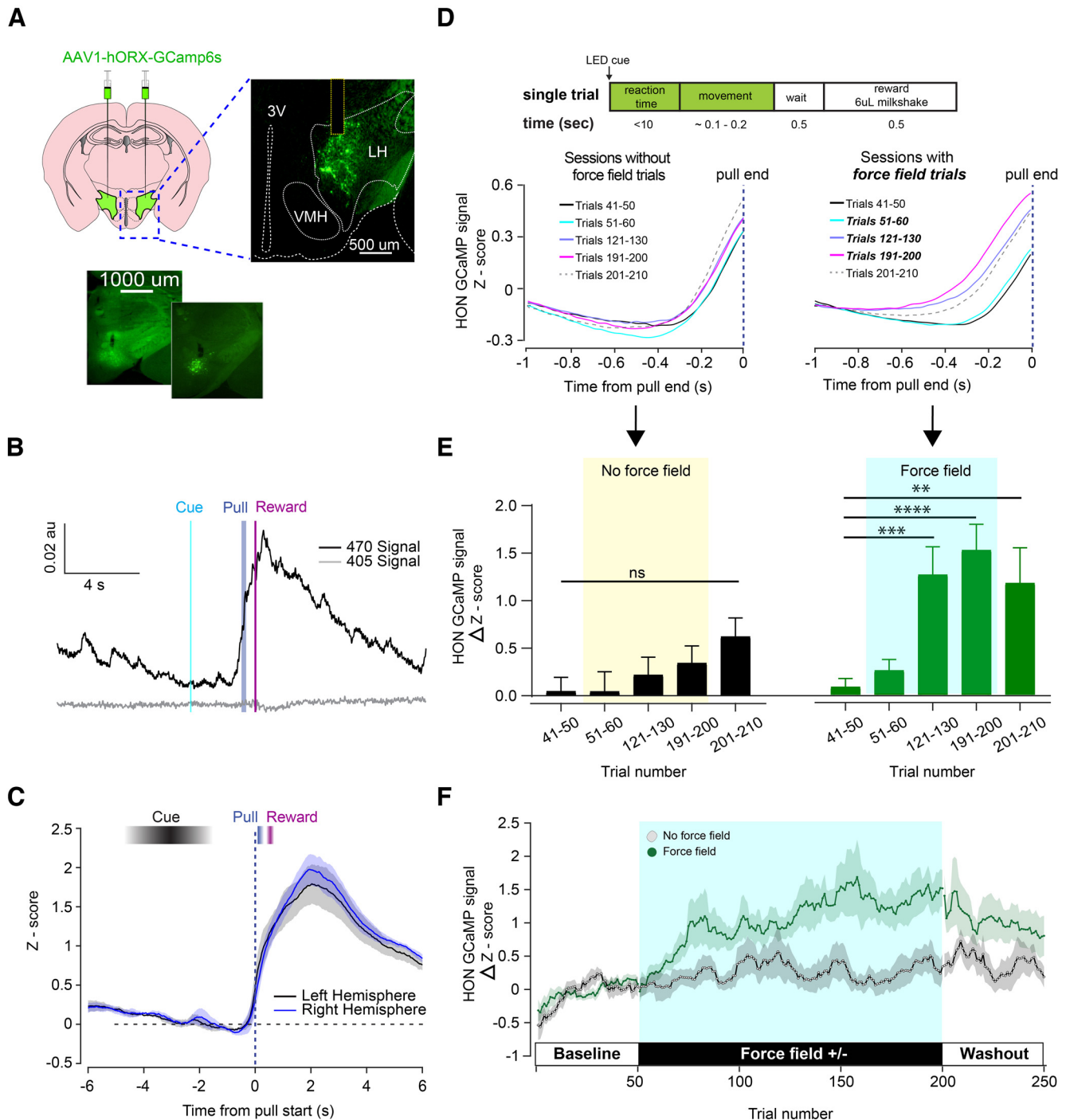
#### Probability of counterforce correction

For the probability of counterforce correction, trials were classified into two classes: with or without counterforce correction (Fig. 6B). The criteria for assigning a trial as a counterforce correction was as follows: if at any point in the trajectory the x-velocity component was higher than a threshold of 10 mm/s in the direction of the force field (empirically determined) and then the x-velocity later switched direction against the force field, this was considered a counterforce correction.

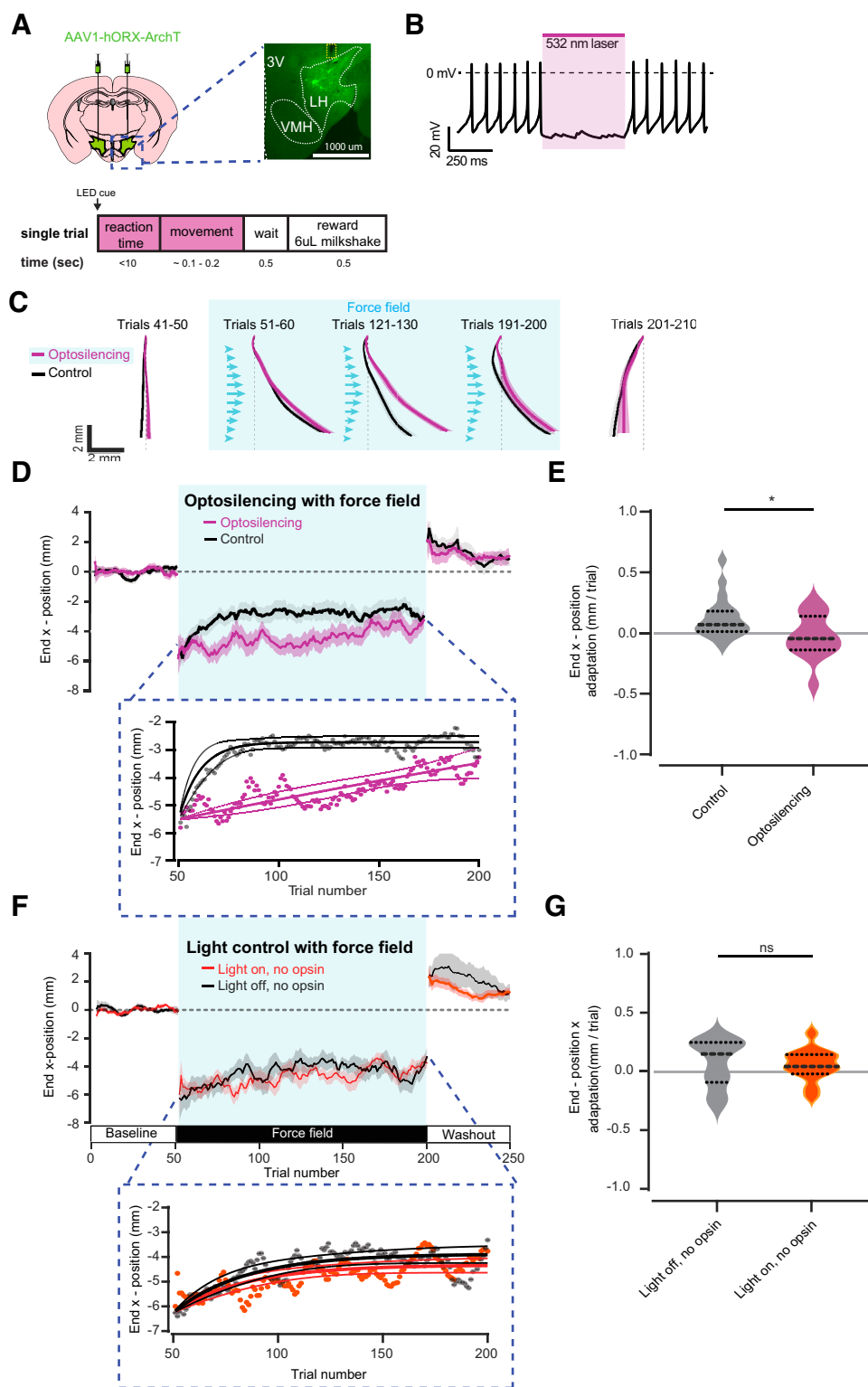
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tests: \*\*\*\* $p < 0.0001$ ,  $ns = p > 0.05$ . **H**, Left panel, Same as in **F**, left panel, showing max y-velocity,  $ns = p > 0.05$ . Right panel, Same as in **F**, right panel, for max y-velocity. RM ANOVA  $F_{(4,128)} = 3.578$ ,  $p < 0.01$ ; Sidak's multiple comparison tests:  $ns = p > 0.05$ . **I**, Left panel, Same as in **F**, left panel, for percentage of successful trials. Right panel, Same as in **F**, right panel, for percentage of successful trials. RM ANOVA  $F_{(4,128)} = 5.532$ ,  $p < 0.001$ ; Sidak's multiple comparison tests:  $ns = p > 0.05$ , \*\*\*\* $p < 0.0001$ .

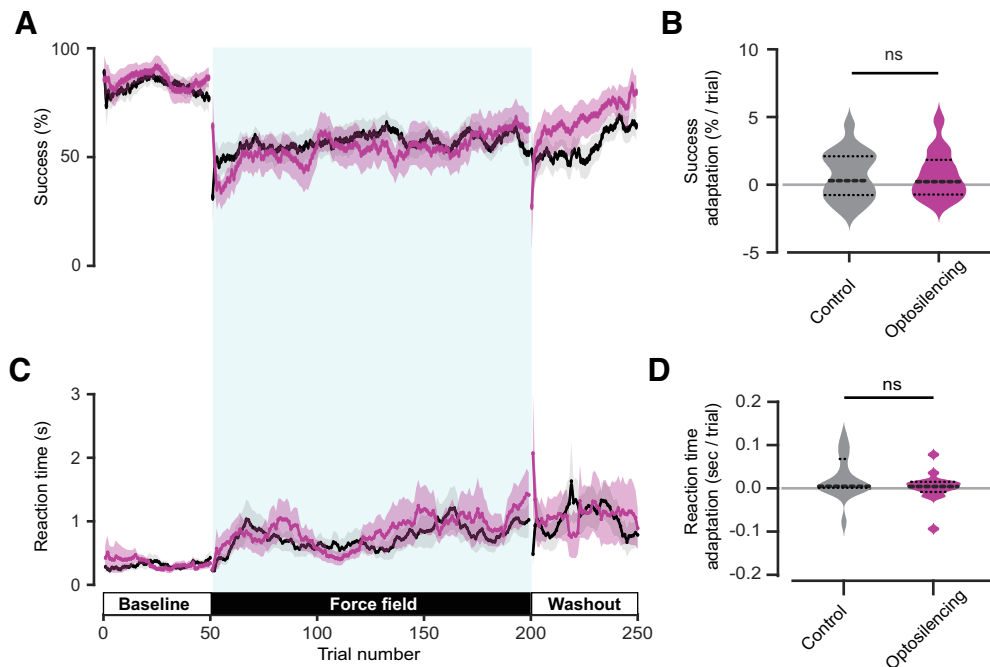




**Figure 2.** HON signals associated with skilled forelimb movements and motor adaptation. **A**, Targeting schematic (left) and typical expression (right) of AAV1-hORX-GCaMP6s in HONs, and example fiber placements in two additional mice (bottom). Dashed yellow box indicates fiber placement. V3, third ventricle; VMH, ventromedial nucleus of the hypothalamus; LH, lateral hypothalamus. **B**, Typical example of raw  $\text{Ca}^{2+}$ -dependent (470 = 470 nm-excited fluorescence) and  $\text{Ca}^{2+}$ -independent (405 = 405 nm-excited fluorescence) components of HON-GCaMP6s signal during trials. Epochs of the single trial are labeled: light blue line indicates the cue onset, blue bar indicates the pull, and magenta line indicates reward delivery. **C**, Z-scored HON-GCaMP6s activity of left (black) and right hemispheres (blue) during nonforce field trials with an intertrial interval of  $>15$  s. Cue bar (gray) indicates the range of trial cue onsets across animals. Blue bar indicates the range of pull durations across animals, magenta bar indicates the range of reward delivery times. Data are mean  $\pm$  SEM for  $n = 50$  trials from 4 mice. **D**, Top, Diagram explaining the window of analysis of premotor and motor-related HON-GCaMP6s signals of a single trial (green bar). Bottom, Evolution of pull-associated HON-GCaMP6s signals across control nonforce field trials (left), and force field trials (right), aligned to pull completion. Traces are baseline subtracted from  $-2$  to  $-1$  s before pull start and averaged across sessions ( $N_{\text{Force field}} = 15$  sessions,  $N_{\text{No Force field}} = 9$  sessions, from 4 mice). **E**, Average GCaMP6s signal amplitude, expressed as  $\Delta Z$ -score, within indicated trial blocks from **D** of no force field (black) and force field (green) sessions. Two-way mixed-effects model analysis, interaction  $F_{(4,56)} = 8.082$ ,  $p < 0.0001$ ; Sidak's multiple comparison tests: \*\*\*\* $p < 0.0001$ , ns =  $p > 0.05$ . Signal trends across the force field trials (cyan shading) and equivalent no force field trials (yellow shading) are compared in Results. **F**, Average GCaMP6s signal amplitude versus trial number across force field (green) and no force field sessions (black),  $\Delta Z$ -score represents signal amplitude (see Materials and Methods;  $N_{\text{Force field}} = 15$  sessions,  $N_{\text{No Force field}} = 9$  sessions, from 4 mice).



**Figure 3.** HON optosilencing impairs motor adaptation. **A**, Top, Targeting schematic (left) and typical expression of ArchT (right) in HONs. Bottom, Single trial diagram depicting when laser stimulation was applied (magenta bar). **B**, Whole-cell patch-clamp brain slice recording confirming optosilencing of an HON-ArchT neuron by green laser (representative example of  $n = 20$  cells). **C**, Average trajectories of baseline, force field, and washout trajectories in control and optosilencing sessions. Blocks of 10 trials were averaged within a session and then averaged across sessions. Means  $\pm$  SEM of 19 control and 14 optosilencing sessions from 7 mice. **D**, Average end x-position value versus trial number. Means  $\pm$  SEM of 19 control (black) and 14 optosilencing (magenta) sessions from 7 mice. Inset, Exponential fits to the data (thick lines) and their confidence intervals (thin lines) demonstrating reduced adaptation rate in control versus optosilenced sessions (extra sum-of-squares  $F$  test of whether  $k$  fit parameters are different between datasets,  $F_{(1,4801)} = 14.08$ ,  $p < 0.001$ ). Smoothed means are shown for visualization only, the fit was performed using individual trial points of unsmoothed data. **E**, For data shown in **D**, initial adaptation rates from individual sessions, obtained using least-squares linear fits of the first 20 force field trials of each session. Violin plot of density, dashed line at median and dotted line at the quartiles, two-tailed Mann–Whitney test,  $U_{(N_{\text{control}} = 19, N_{\text{optosilenced}} = 14)} = 73$ ,  $*p < 0.05$ . **F**, Average end x-position value versus trial number of light control mice. Means  $\pm$  SEM of 10 control (black) and 13 light on (orange) sessions from 6 mice. Inset, Exponential



**Figure 4.** HON optosilencing does not impair reward rates or motivational metrics. **A**, Average percentage of successful trials versus trial number of control and HON optosilencing sessions. Means  $\pm$  SEM of 19 control and 14 optosilencing sessions from 7 mice. **B**, For data shown in **A**, initial adaptation rates from individual sessions, obtained using least-squares linear fits of the first 20 force field trials of each session. Violin plot of density, dashed line at median and dotted line at the quartiles, Mann–Whitney test,  $U_{(N_{\text{control}} = 19, N_{\text{optosilenced}} = 14)} = 131$ , ns =  $p > 0.05$ . **C**, As in **A** for reaction time, calculated as the time between cue onset and pull onset. **D**, As in **B** for data in **C**, Mann–Whitney test,  $U_{(N_{\text{control}} = 19, N_{\text{optosilenced}} = 14)} = 105$ , ns =  $p > 0.05$ .

#### Steering angle

The initial steering angle was calculated from the x-position ( $X_{0.5}$ , in mm) corresponding to y position of 0.5 mm by finding the arctan of  $X_{0.5}/0.5$ , and shown in degrees from y-axis (Figs. 1C,G, 6C–E). Trials in which the total distance covered during the pull was  $<2$  mm were discarded from analyses. Since preoptointerference behavioral state could affect the impact of optointerference, we ensured that control and optointerfered sessions had comparable preoptointerference motor outputs by discarding from analysis any sessions where the mean steering angle in the first 50 trials (i.e., preforce field, preoptointerference) was above or below  $25^\circ$ . In the analyzed sessions, individual data points of end x-position and angle were then baselined to the mean of the first 50 trials, for clearer visualization of the change from preforce field trials.

#### Success

Success was defined as a correctly executed trial/pull which was rewarded with milkshake. The % success (Figs. 1I, 4A) was computed from the sliding average of 10 trials in a session.

#### Missed trials, force field session time, and reaction times

Missed trials were defined as the % of trials during which the mouse did not attempt to pull for 10 s after the cue. Force field session time was defined as the total time taken to complete the 150 force field trials. Within-trial reaction time was defined as the time between the cue onset and time when the mice initiated the pull (pull start). All variables plotted as a function of trial number were smoothed using a 10 trial moving mean within a session before averaging sessions (Figs. 1F–I, 2E, 3D,F,

4A,C, 6A–F). This was done for visualization only; for statistical data analysis, unsmoothed data were used.

#### Number of trials to reach 80% max adaptation

Force field trials in individual sessions were analyzed. Each session was smoothed using the 10 trial moving average, then the 80% value of the maximal end x-position was noted. Then, the trial number needed to reach the 80% value was determined by finding the first trial above the 80% criterion.

#### Computational models of sensory prediction error-based and reward prediction error-based motor adaptation

Based on previous work (Todorov and Jordan, 2002; Berniker and Kording, 2008; Izawa and Shadmehr, 2011; Mathis et al., 2017), we used a sensory prediction error-based model and a reward prediction error-based model to quantify the influence of those two factors on motor adaptation.

#### Sensory prediction error-based model

Specifically, the end x-position  $x_k$  at trial  $k$  was modeled as being dependent on the motor command  $u_k$ , the perturbation  $p_k$ , and motor noise  $\eta_m \sim N(0, \sigma_m)$ :

$$x_k = u_k + p_k + \eta_m.$$

Further, the approach assumes that mice receive an estimate of their paw position through proprioception that is influenced by sensory noise  $\eta_s \sim N(0, \sigma_s)$ :

$$y_k = x_k + \eta_s.$$

Given the efference copy of the motor command, mice can provide an internal estimate of their expected paw position:

←

fits to the data (thick lines) and their confidence intervals (thin lines), demonstrating no difference in adaptation rate because of brain heating (extra sum-of-squares  $F$  test of whether  $k$  fit parameters are different between datasets,  $F_{(1,3378)} = 3.330$ ,  $p > 0.05$ ). **G**, Same as in **E**, for light controls. Violin plot of density, dashed line at median and dotted line at the quartiles, two-tailed Mann–Whitney test,  $U_{(N_{\text{LightOn}} = 13, N_{\text{LightOff}} = 10)} = 58$ ,  $p > 0.05$ .

$$\hat{x}_k = \hat{p}_k + u_k$$

relies on an internal model of the perturbation  $\hat{p}_k$  that is obtained over trial to trial and is updated based on a retention factor  $r$  ( $0 \leq r \leq 1$ ) and noise  $\eta_p \sim N(0, \sigma_p)$ :

$$\hat{p}_{k+1} = r\hat{p}_k + \eta_p.$$

These relationships can be expressed in matrix form within a Kalman filter framework (Kalman, 1960):

$$X_{k+1} = AX_k + Bu_k + \eta,$$

with the states  $X_k = \begin{pmatrix} p_k \\ x_k \end{pmatrix}$ , the state-transition matrix  $A = \begin{pmatrix} r & 0 \\ 1 & 0 \end{pmatrix}$ , the control-input vector  $B = \begin{pmatrix} 0 \\ 1 \end{pmatrix}$ , and noise  $\eta_p \sim N(0, Q)$  with  $Q = \begin{pmatrix} \sigma_p^2 & 0 \\ 0 & \sigma_m^2 \end{pmatrix}$ . In addition, the observation equation is defined as:

$$y_k = HX_k + \eta_s,$$

with the observation model  $H = \begin{pmatrix} 0 & 1 \end{pmatrix}$ . Based on this Kalman filter framework (details in Todorov and Jordan, 2002; Berniker and Kording, 2008; Izawa and Shadmehr, 2011; Mathis et al., 2017), an a-priori prediction of the paw position can be made, which is compared with the obtained sensory feedback and then used to update the internal model of the perturbation (i.e., sensory prediction error-based learning). Given this internal model of the perturbation, the optimal motor command to counteract the perturbation is:

$$u_k = -\hat{p}_k.$$

#### Reward prediction error-based model

In addition, it is assumed that reward might influence motor adaptation. Hence, reward prediction error-based motor adaptation was modeled using an actor-critic reinforcement learning approach based on the temporal difference rule (Todorov and Jordan, 2002; Berniker and Kording, 2008; Izawa and Shadmehr, 2011; Mathis et al., 2017). Specifically, the optimal action  $a_k$  according to this model was defined as:

$$a_{k+1} = a_k + \alpha_a \delta,$$

with the learning rate  $\alpha_a$  ( $\alpha_a > 0$ ) and the reward prediction error  $\delta$  being defined as:

$$\delta = f_k + \gamma \hat{V}_{k+1} - \hat{V}_k,$$

with a discounting factor  $\gamma$  ( $0 \leq \gamma \leq 1$ ), the value function  $\hat{V}_k$ ,

$$\hat{V}_{k+1} = \hat{V}_k + \alpha_v \delta,$$

and the reward function  $f_k$ :

$$\begin{aligned} \text{if trial } k \text{ is rewarded: } f_k &= 1 - \beta u_k^2, \\ \text{if trial } k \text{ is not rewarded: } f_k &= -\beta u_k^2, \end{aligned}$$

with the cost parameter  $\beta$ .

#### Fitting of models to experimental data

We fit two separate models to the data, the first purely relying on the reward prediction error to update motor command, and the second purely relying on the sensory prediction error:

$$\text{Reward prediction error – based model: } u_k = a_k + \eta_e,$$

$$\text{Sensory prediction error – based model: } u_k = -\hat{p}_k$$

with exploratory noise  $\eta_e \sim N(0, \sigma_e)$ . In each case, the model was fitted to minimize the root mean square error between the estimated and the actual end x-position of mice in the control condition during the force field. For each mouse, the mean of the baseline data were subtracted.

Further, a perturbation  $p_k$  was defined for each mouse based on the average value in end x-position during the first three trials in the force field. In addition, the end x-position was used for the proprioception-based estimate of the paw position  $y_k$ .

The free parameters of the model were optimized through a pattern search algorithm (MATLAB function pattern search). Given that this optimization procedure requires the random initialization of free parameters that affects the optimization results, the optimization process was repeated 50 times with different initial parameter values. For the model purely based on the sensory prediction error, the free parameters were optimized on the following intervals:  $r \in [0, 1]$ ,  $\sigma_m \in [0, \tau]$ ,  $\sigma_s \in [0, \tau]$ ,  $\sigma_p \in [0, \tau]$ , where  $\tau$  was defined as the mean interquartile range of end x-position during the baseline period. For the model purely based on the reward prediction error, the free parameters were randomly initialized and optimized on the following intervals:  $\sigma_m \in [0, \tau]$ ,  $\sigma_e \in [0, \tau]$ ,  $\alpha_e \in [0, 1]$ ,  $\alpha_a \in [0, 1]$ ,  $\gamma \in [0, 1]$ ,  $\beta \in [0, 1]$ . For both models, all parameters were randomly initialized on the interval  $[0, 1]$ . For the 50 optimizations of the reward prediction error-based model, 11 optimization runs had to be removed because of the occurrence of extreme values (infinity) in the model output. No optimization runs had to be removed for the sensory prediction error-based model. In Figure 5, we visualized the motor commands averaged across these realizations. For Figure 5B,D, we report the strength of the command  $u_k$  from the sensory-based and reward-based models separately. Additionally, we normalized  $u_k$  relative to the sensory prediction error  $y_k - HX_k$  and the reward prediction error  $\delta$  for the sensory-based and reward-based models, respectively. This allowed to capture the magnitude of model contributions relative to the changes in sensory perception or reward rate, thereby allowing better distinction between the contribution of different modalities.

#### Statistical analyses

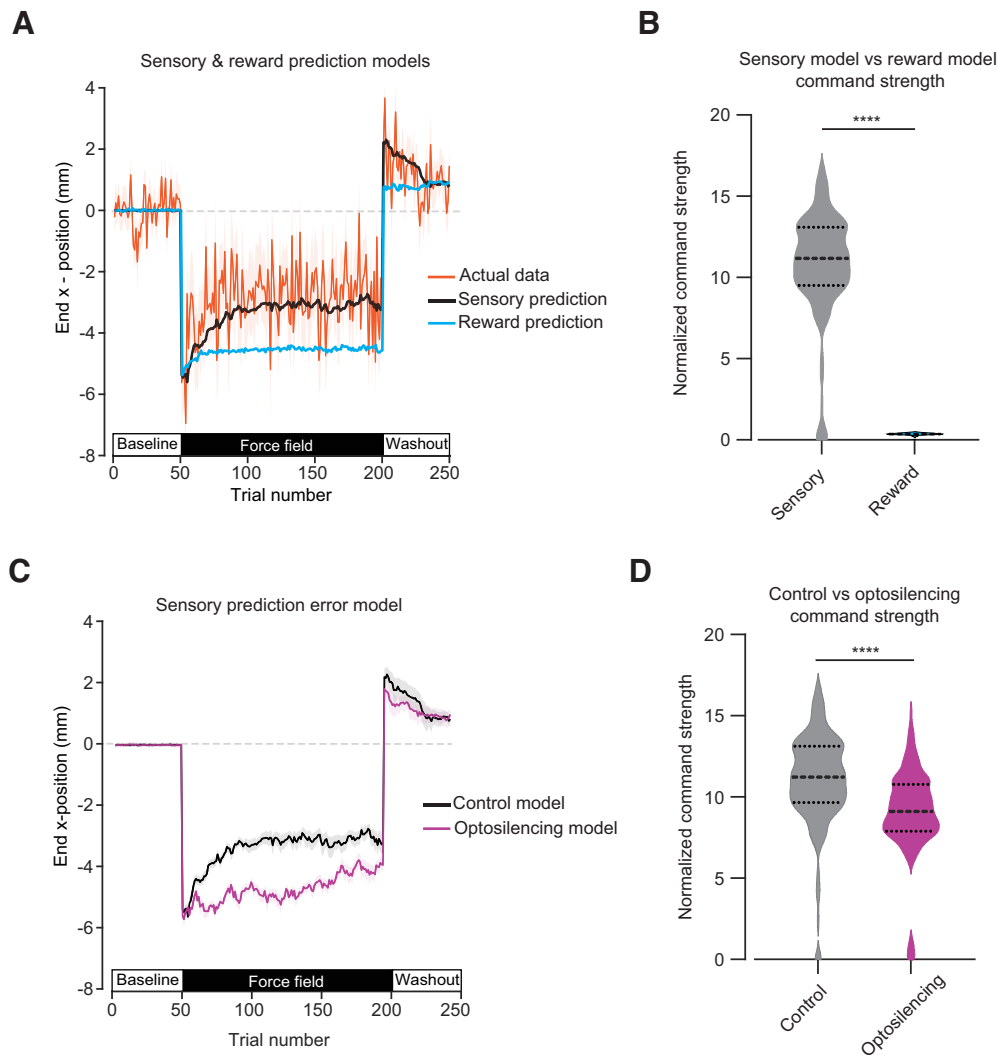
Statistical analyses were performed using Prism 8.4.3 (GraphPad Inc.). To test the hypothesis that optogenetic manipulations affect adaptation rate of various parameters (end x-position, angle, velocity, reward rate, within-trial reaction times), we first examined the slopes of least-squares linear fits of the plots of the parameter and trial number in the first 20 force field trials, where the trajectory error-based adaptation was expected to be the greatest because of the greatest error at the start of the force field, and before adaptation of control sessions reached a plateau (from visual examination of control end x-position adaptation in Fig. 3D). If the difference in these slopes in control and optomanipulated sessions was significant (Figs. 3E, 6C), we then assessed adaptation rates of the full 150 force field trials using exponential fit statistics; and if it was not significant (Figs. 3G, 4B,D, 6A,B,E,F), the rates were not analyzed further. Where exponential fit comparisons were performed (Figs. 3D,E, 6C,D), the adaptation rates were estimated using a least-squares fit on an exponential plateau equation to all data points including all sessions and all trials in the 150 force field trial block (trials 50–200) of each dataset. The equation was:  $Y = Y_M - (Y_M - Y_0) \cdot \exp(-k \cdot x)$ , where  $Y$  is end x-position or angle,  $Y_M$  is the plateau value,  $Y_0$  is the initial value, and  $k$  is adaptation rate.  $Y_0$  values were estimated from the mean of the first five force field trials and fixed as shared parameters between control and optosilenced datasets, and  $Y_M$  and  $k$  were free parameters during the fits. An extra sum-of-squares  $F$  test was then used to assess whether the best-fit values of  $k$  differed between datasets. Group data are presented as mean  $\pm$  SEM unless indicated otherwise.

## Results

### Reproducing assessment of human upper limb adaptation using mouse-robot interactions

We first explored whether mice can perform complex and precisely quantifiable forelimb tasks that mimic those used in robot-assisted studies of human upper limb adaptation (Shadmehr and Mussa-Ivaldi, 1994). A total of 18 mice were trained to pull a robotic arm on a defined trajectory (9-mm straight pull with  $<5$ -mm lateral deviation was rewarded with milkshake, Fig. 1A, B; see Materials and Methods). The robot tracked pull dynamics



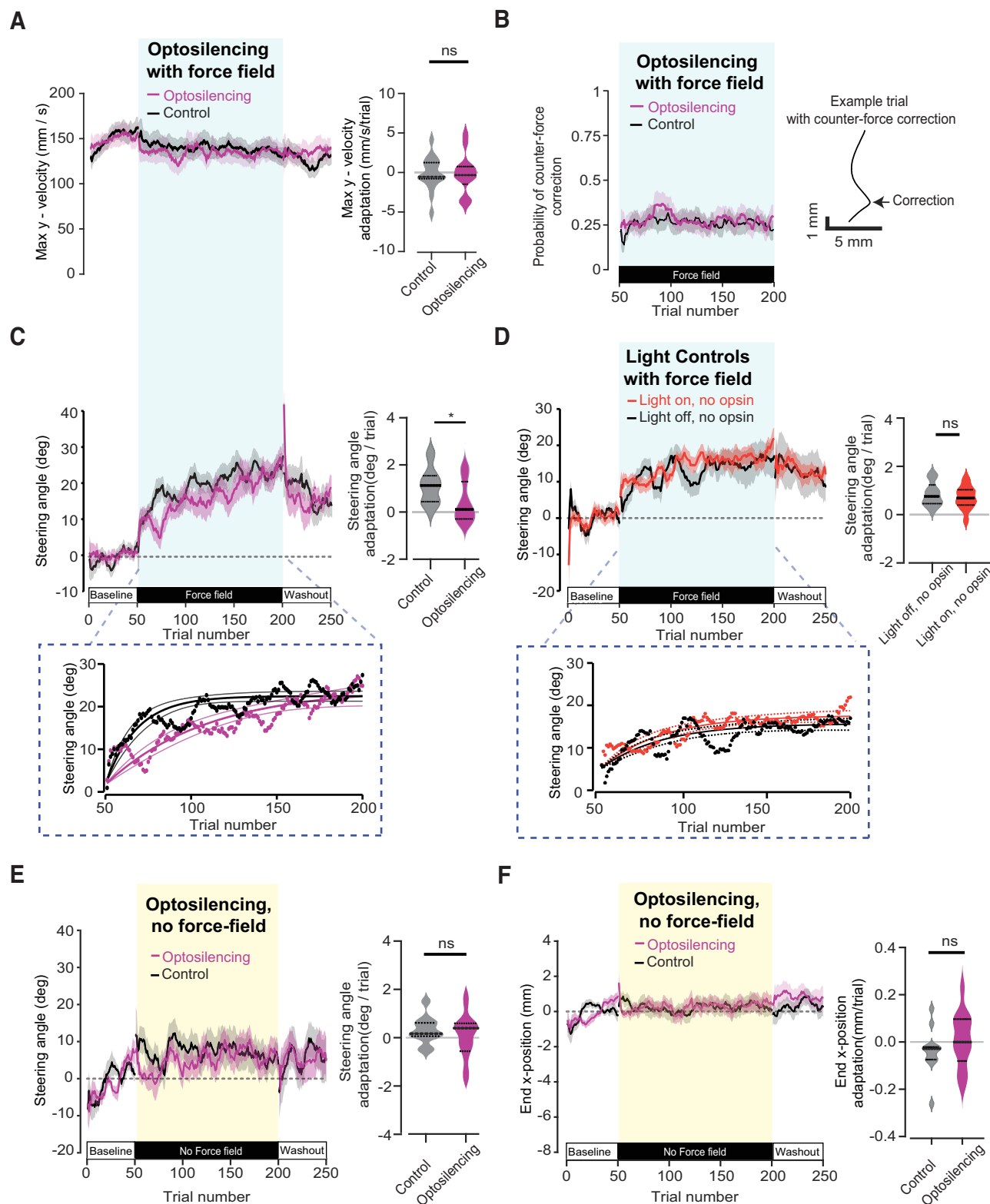


**Figure 5.** Forelimb adaptation and effects of HON optosilencing follow a sensory prediction error-based model. **A**, Orange line shows the average end x-position value versus trial number for control force field data from 19 sessions. The shaded orange area depicts the SEM. Black line depicts a sensory prediction error-based model that was fitted to the force field data of each session. The blue line depicts the reward prediction error-based model. The average of 50 realizations of the model with different random initial parameters is visualized. The sensory prediction error-based model shows population-level root-mean-square error of the fit is 1.12 cm, and the reward prediction error-based model shows population-level root-mean-square error of the fit is 1.98 cm. **B**, Normalized command strength contributions of the reward prediction error-based model (blue) and the sensory prediction error-based model (black) of control sessions. The command strength of the sensory and reward-based models were normalized with respect to the sensory and reward prediction error, respectively. Violin plot of density, dashed line at median and dotted line at the quartiles, two-tailed Mann–Whitney test,  $U_{(N_{\text{control}} = 152, N_{\text{optosilenced}} = 156)} = 737$ , \*\*\*\* $p < 0.0001$ . **C**, As in **A** for only the sensory prediction error-based model for both control and optosilencing sessions. **D**, As in **B** for the normalized contributions of sensory prediction error-based model for both control and optosilencing sessions. Violin plot of density, dashed line at median and dotted line at the quartiles, two-tailed Mann–Whitney test,  $U_{(N_{\text{control}} = 152, N_{\text{optosilenced}} = 156)} = 6295$ , \*\*\*\* $p < 0.0001$ .

and kinematics at a high temporal resolution, and used this information to compute and impose precisely defined velocity-dependent force fields perpendicular to the pulling motion. This perturbed the pull trajectory in real-time (Fig. 1*B–E*). The pull was performed in the dark, so sensory feedback was limited to somatosensation. After the mice performed 50 pulls without a force field (baseline trials), the robot introduced a velocity-dependent force field for the next 150 pulls (force field trials), followed by 50 pulls without the force field (washout trials; Fig. 1*B–E*). During initial force field trials, the unpredicted force field produced significant lateral displacement of the pull trajectory, quantified using the lateral endpoint position (“end x-position,” explanation in Fig. 1*C,D–F*; statistical analysis in Fig. 1*F*, right panel). During subsequent trials, mice progressively compensated for the force field (Fig. 1*D–F*), reducing lateral displacement by ~50% by the end of the 150 force field trials [Fig. 1*F*, right panel, one-way repeated measures (RM) ANOVA across

force field trials,  $F_{(2,64)} = 13.53$ ,  $p < 0.0001$ ]. In the washout trials, the unpredicted removal of the force field resulted in a significant x-overshoot in the direction opposing the force field (Fig. 1*D–F*; statistical analysis in Fig. 1*F*, right panel).

In theory, mice had several ways to reduce the perturbation induced by the velocity-dependent force field, including reducing pull velocity and/or adapting the initial pull angle to counteract the force field. Across force field trials, mice progressively changed their initial pull angle, measured during the first 0.5 mm of the pull, i.e., before force field onset (Fig. 1*G*, right panel; one-way RM ANOVA across force field trials,  $F_{(2,64)} = 25.09$ ,  $p < 0.0001$ ; steering angle explanation in Fig. 1*C*). This preemptive steering indicates that mice learnt to expect the force field from recent force field trials, and predictively deployed compensatory motor commands. The delay in resuming preforcefield pull angles in washout trials confirmed this (Fig. 1*G*, right panel). In contrast, we found no significant within-force field adaptation



**Figure 6.** HON optosilencing disrupts sensory prediction error-based model through impaired sensorimotor integration. **A**, Left panel, Average max y-velocity versus trial number. Means  $\pm$  SEM of  $n = 19$  force field sessions and 14 optosilencing sessions from 7 mice. Right panel, Initial adaptation rates from individual sessions for data shown in left panel, obtained using least-squares linear fits of the first 20 force field trials of each session. Violin plot of density, dashed line at median and dotted line at the quartiles, two-tailed Mann–Whitney test,  $U_{(N_{\text{Control}} = 19, N_{\text{Optosilenced}} = 14)} = 129$ ,  $ns = p > 0.05$ . **B**, Average probability of a trial having a counterforce feedback correction versus trial number. Inset describes an example trial with counterforce feedback correction (for calculation, see Materials and Methods). **C**, Left panel, Same as **A**, but for steering angle. Inset, Exponential fits to the data (thick lines) and confidence intervals (thin lines), illustrating reduced adaptation rate (extra sum-of-squares  $F$  test of whether  $k$  fit parameters are different between datasets,  $F_{(1,4797)} = 13.49$ ,  $p < 0.001$ ). Smoothed means are shown for visualization only, the fit was performed using individual trial points of unsmoothed data. Right panel, Initial adaptation rates from individual sessions for data shown in **A**, obtained using least-squares linear fits of the first 20 force field trials of each session. Violin plot of density, dashed line at median and dotted line at the quartiles, two-tailed Mann–Whitney test,  $U_{(N_{\text{Control}} = 19, N_{\text{Optosilenced}} = 14)} = 70$ ,  $*p < 0.05$ . **D**, Left panel, Same as in **A**, left panel, for light control mice, demonstrating no difference in adaptation rate because of tissue heating (extra sum-of-squares  $F$  test of whether  $k$  fit parameters are different between datasets,  $F_{(1,3372)} = 0.9682$ ,

in y-velocity (Fig. 1H, right panel; one-way RM ANOVA across force field trials,  $F_{(2,64)} = 3.021$ ,  $p > 0.05$ ).

Importantly, the reward rate did not change significantly during force field trials (Fig. 1I, right panel; one-way RM ANOVA across force field trials,  $F_{(2,64)} = 1.895$ ,  $ns = p > 0.05$ ). Such absence of a strong relationship between trial-to-trial changes in motor output and reward feedback is thought to indicate that the observed motor adaptation is driven primarily by sensory errors rather than reward errors (Izawa and Shadmehr, 2011; Mathis et al., 2017).

Cumulatively, these data indicate that mice adapted to the velocity-dependent force field by updating the sensory-error-based internal model, manifested by (1) change in the initial pull angle, (2) progressively corrected x-displacement across the force field trials, (3) significant after effects when the force field was removed.

### HON activity associated with task execution and motor adaptation

The development of the human-like adaptation task for mice (Fig. 1) allowed us to apply neural sensing technologies to explore HON correlates of skilled forelimb movements and motor adaptation. Genetic targeting of the calcium sensor GCaMP6s to HONs can be used to observe their activity at high temporal resolution (Karnani et al., 2020; Fig. 2A). Measuring emitted GCaMP6s fluorescence in these cells is a good proxy for activity, because it is linearly related to HON action potential firing frequency (González et al., 2016b). Anatomically, HONs show ~80% ipsilateral preference in projecting to motor-related targets such as the cortex and spinal cord (van den Pol, 1999; Jin et al., 2016). However, these targets control contralateral and ipsilateral limbs, respectively, and so unilateral HONs may in theory influence both forelimbs. Consistent with this, when we recorded HON activity bilaterally, we found no clear difference between movement-associated signals between hemispheres (Fig. 2C), and therefore combined data from both hemispheres in subsequent analyses. Recording HON-GCaMP6s activity during pull execution revealed that each pull was associated with a distinct rise in GCaMP6s fluorescence that became apparent ~400 ms before the pull onset, persisted throughout the pull, and lasted for a few seconds afterward (Fig. 2B,C). To control for movement artifacts in these recordings, we examined the raw calcium-independent HON-GCaMP6s fluorescence signal evoked by isosbestic 405-nm excitation (Kim et al., 2016). During the pulls, we did not observe similar increases in 405 nm-evoked fluorescence (Fig. 2B). This confirms that the HON-GCaMP6s signals are not movement artefacts, but reflect endogenous hypothalamic HON activity waves temporally-associated with execution of skilled forelimb movements (HONWEMs).

We next examined whether HONWEMs change during motor adaptation. Formal frameworks of motor adaptation

envision that motor commands are updated to reduce sensory errors (Wolpert et al., 1998; Izawa and Shadmehr, 2011). During force field adaptation, we thus reasoned that a neural signal representing sensory errors would initially be high, then decay during error-reducing adaptation. In turn, a signal representing components of motor adaptation would gradually increase during force field adaptation, whereas a signal not involved, such as a permissive signal for movement, would stay constant.

To differentiate between these possibilities, we tracked HONWEM amplitude (quantified at movement end) across baseline, force field, and washout trials. We found that HONWEM amplitude gradually increased during force field trials (Fig. 2E, one-way RM ANOVA across force field trials,  $F_{(2,58)} = 20.74$ ,  $p < 0.0001$ ; Fig. 2F). In contrast, when the same experiment was performed in the same mice without the force field, HONWEM amplitude was unchanged across the trial epoch that corresponded to force field trials in force field sessions (Fig. 2E, one-way RM ANOVA across trials 51–200,  $F_{(2,34)} = 1.182$ ,  $p > 0.05$ ; Fig. 2F). The latter finding confirms that the gradual increase in HONWEM amplitude during force field trials was related to responses to force field, rather than to nonspecific factors such as task duration or satiation. Overall, our data therefore suggest that HONWEMs represent some aspect(s) of updated motor command, rather than a sensory error signal.

### Role of movement-associated HON signals in forelimb motor adaptation

To explore whether HONWEMs influence adaptation rather than simply represent it, we used optogenetics to inhibit them during force field adaptation. We targeted the optogenetic inhibitory opsin ArchT to HONs (Garau et al., 2020; Karnani et al., 2020), and bilaterally implanted optic fibers in the lateral hypothalamus (Fig. 3A). We confirmed effective HON-ArchT optosilencing using patch-clamp recordings in acute brain slices (Fig. 3B). On each force field trial, lateral hypothalamic illumination (bilateral 532-nm laser) started at the cue and stopped at the end of the pull before reward delivery (see Materials and Methods), thus targeting premotor and movement epochs of each pull, since both of these epochs are implicated in movement control (da Silva et al., 2018; Karnani et al., 2020; Inagaki et al., 2022).

The HONWEM-optosilenced force field sessions displayed significantly slower rates of motor adaptation compared with control force field sessions in the same mice [trajectory examples, Fig. 3C,D, extra sum-of-squares  $F$  test comparison of exponential rate constants across all 150 force field trials:  $F_{(1,296)} = 18.33$ ,  $p < 0.0001$ , Fig. 3E; comparison of linear slopes of the initial 20 force field trials, chosen as the region with the steepest adaptation before control sessions reached plateau, see Materials and Methods, Mann–Whitney test,  $U_{(N_{\text{control}} = 19, N_{\text{optosilenced}} = 14)} = 73$ ,  $p = 0.0288$ ; Fig. 3E]. Additionally, during the force field trial block, we quantified the number of trials to reach 80% of maximal adaptation in each session (see Materials and Methods), and found that optosilencing increased this number, indicating slower adaptation (optosilenced:  $100.4 \pm 12.8$  trials, control:  $62.1 \pm 8.8$  trials, two-tailed Mann–Whitney test,  $U_{(N_{\text{control}} = 19, N_{\text{optosilenced}} = 14)} = 68.5$ ,  $p = 0.017$ ).

Of note, the different adaptation rates in HONWEM-optosilenced and control force field sessions involved similar initial trajectory displacements, and thus similar sensory errors, during the first force field trial (Fig. 3D; trial 51: optosilenced end x-position:  $-5.8 \pm 1.17$  mm, control:  $-5.62 \pm 1.37$  mm,  $n = 14$  and 19 sessions from 7 mice, respectively; two-tailed Mann–

←  
 $p > 0.05$ ). Right panel, Same as (A, right panel) for data shown in left panel, two-tailed Mann–Whitney test,  $U_{(N_{\text{lightOff}} = 10, N_{\text{lightOn}} = 13)} = 61$ ,  $ns = p > 0.05$ . E, Left panel, Average steering angle value versus trial number, without the force field. Means  $\pm$  SEM of 11 control and 13 optosilencing sessions from 7 mice. Right panel, As in A, right panel, for data in left panel, two-tailed Mann–Whitney test,  $U_{(N_{\text{control}} = 11, N_{\text{optosilenced}} = 13)} = 64$ ,  $ns = p > 0.05$ . F, Left panel, Average end x-position value versus trial number, without the force field. Means  $\pm$  SEM of 11 control and 13 optosilencing sessions from 7 mice. Right panel, As in A, right panel, for data in left panel, two-tailed Mann–Whitney test,  $U_{(N_{\text{control}} = 11, N_{\text{optosilenced}} = 13)} = 53$ ,  $ns = p > 0.05$ .

Whitney test,  $U_{(N_{\text{control}} = 19, N_{\text{optosilenced}} = 14)} = 132$ ,  $p > 0.05$ ). Importantly, this indicates that the force field initially perturbed limb movements to a similar extent during control and optosilenced trials, but that optosilencing impaired motor updating in response to the sensory error. Despite this slower updating of motor commands in HONWEM-optosilenced sessions, at the end of the 150 consecutive force field trials the mice reached similar levels of motor output to control force field sessions (Fig. 3D). Thus, optosilencing did not abolish the ability of the motor system to eventually generate control-like trajectories, but selectively affected the rate of trajectory updating. The same optomanipulation in control mice (without inhibitory opsins) did not influence forelimb adaptation, confirming that motor adaptation impairment (Fig. 3C–E) was not because of artefacts such as tissue heating (Fig. 3F, extra sum-of-squares  $F$  test of whether  $k$  fit parameters are different between datasets,  $F_{(1,3378)} = 3.330$ ,  $p > 0.05$ ; Fig. 3G, two-tailed Mann–Whitney test,  $U_{(N_{\text{LightOff}} = 10, N_{\text{LightOn}} = 13)} = 58$ ,  $p > 0.05$ ).

### Role of movement-associated HON signals in general task engagement

In contrast to these differences in motor adaptation rate, in HONWEM-optosilenced and control force field sessions, mice displayed similar reward success rates (Fig. 4A,B), similar cue-to-pull-initiation times (Fig. 4C,D), similar numbers of missed trials (% of trials where pull was not initiated in  $>10$  s after cue: control =  $5.26 \pm 3.10$ ; optosilenced =  $6.43 \pm 2.43$ , two-tailed Mann–Whitney test,  $U_{(N_{\text{control}} = 19, N_{\text{optosilenced}} = 14)} = 87.50$ ,  $p > 0.05$ ), and similar times to complete the 150 force field trials (control =  $20.14 \pm 1.209$  min; optosilenced =  $20.34 \pm 1.000$  min, two-tailed Mann–Whitney test,  $U_{(N_{\text{control}} = 19, N_{\text{optosilenced}} = 14)} = 115$ ,  $p > 0.05$ ). Collectively, these results suggest that the HONWEMs were not required for the general motivation or attentiveness to perform the task.

### HON optosilencing results in impaired sensory prediction error-based adaptation

Recent evidence has shown that motor adaptation can be driven by both sensory-prediction errors and reward prediction errors (Izawa and Shadmehr, 2011). From previous studies in motor adaptation, internal models are updated to minimize sensory prediction errors, i.e., the error between the sensory prediction and sensory outcome (Oh and Schweighofer, 2019). Alternatively, reward prediction error-based models suggest the internal model of a motor plan would be adapted strictly based on whether actions resulted in receiving a reward. Based on previous work (Todorov and Jordan, 2002; Berniker and Kording, 2008; Izawa and Shadmehr, 2011; Mathis et al., 2017), we used a sensory prediction error-based model and a reward prediction error-based model to quantify the influence of those two factors on motor adaptation. We first tested each model on data from control mice to determine the relative contribution of reward prediction error and sensory prediction error to adaptation across the force field (Fig. 5A,B). Consistent with the incremental increase in the reward rate across the force field, a reward prediction error-based learning model failed to fit the behavioral data, in contrast to a sensory prediction error-based model which accounted well for the observed pattern of motor adaptation (Fig. 5A,B; two-tailed Mann–Whitney test,  $U_{(N_{\text{control}} = 152, N_{\text{optosilenced}} = 156)} = 737$ ,  $p < 0.0001$ ). Based on the previous results, we further evaluated the role of HON optosilencing in the sensory prediction error-based model. We performed the same analysis on optosilenced sessions and found that HON inactivation resulted in impaired

sensory prediction error-based adaptation (Fig. 5C,D; two-tailed Mann–Whitney test,  $U_{(N_{\text{control}} = 152, N_{\text{optosilenced}} = 156)} = 6295$ ,  $p < 0.0001$ ).

### Effect of HON silencing on subprocesses of motor adaptation

We next focused on trying to dissect subprocesses of limb movement that could explain the impairment of the sensory prediction error-based model in HON optosilencing, shown in Figures 3C–E and 5. A few different factors could explain why HON optosilencing impairs the model: (1) reduced strength of motor output; (2) impairment of online sensory perception and thus online motor correction; (3) improper sensorimotor representation of the force field. We performed experiments and analysis aimed at investigating whether such factors may explain the motor adaptation impairment observed in Figure 3C–E.

First, we wanted to determine whether a reduction in strength was responsible for the impaired adaptation to the force field during HON silencing. We looked at the max y-velocity of a trajectory as a proxy for strength, and found no significant difference between control and optosilenced sessions, suggesting HON inhibition did not significantly impair mouse forelimb strength (Fig. 6A; two-tailed Mann–Whitney test,  $U_{(N_{\text{control}} = 19, N_{\text{ArchT}} = 14)} = 129$ , ns =  $p > 0.05$ ). Furthermore, since the force field is velocity-dependent, this suggests that in both control and opto-silencing sessions, mice experienced a similar amount of perturbation force from the robot on a per trial basis, so differences in force field strength cannot account for the differences in the end x-position. Finally, because end x-position in optosilencing trials reached similar levels to controls in the last forcefield trials, this suggests strength needed to create a correction was not impaired (Fig. 3D).

Next, we examined online (i.e., within-trial) corrections, which can be detected by measuring sudden corrections in direction during a pull (example, Fig. 6B, inset; for description, see Materials and Methods), presumably arising from online sensory feedback. The probability of a mouse making such corrections against the force field was unchanged by HON optosilencing (Fig. 6B), suggesting that sensory perception of the force field and motor output (spinal loops, etc.) was not abolished by HON optosilencing.

The internal model represents a model of the neuromuscular system in combination with the external world, and consequently acts as a neural simulator that makes predictions of the effect of the current motor plan (Franklin and Wolpert, 2011). A necessary input for the model is a copy of the current motor plan, known as the efference copy. The efference copy is used as a sensorimotor template to perform sensory state estimation, prediction of sensory feedback, or purely for feedforward (predictive) control outcomes. In the forelimb adaptation task, sensory feedback loops presumably do not play a significant role during the first 0.5 mm of a pull, before the forcefield is engaged. Steering angle during this period can thus be used as a metric to estimate the response of the internal model to the efference copy input. We found that HON optosilencing reduced the rate of adaptation in the steering angle (Fig. 6C, extra sum-of-squares  $F$  test of whether  $k$  fit parameters are different between datasets,  $F_{(1,4797)} = 13.49$ ,  $p < 0.001$ ; Fig. 6D two-tailed Mann–Whitney test,  $U_{(N_{\text{control}} = 19, N_{\text{optosilenced}} = 14)} = 51$ ,  $p < 0.01$ ,  $n = 7$  mice). This effect of optomanipulation was not seen in control mice whose HONs lacked the inhibitory optoactuators (Fig. 6D; two-tailed Mann–Whitney test,  $U_{(N_{\text{LightOff}} = 10, N_{\text{LightOn}} = 13)} = 61$ , ns =  $p > 0.05$ ,  $n = 6$  mice).



These results are consistent with the hypothesis that HONWEMs may be selectively involved in emission of updated motor commands during force field adaptation. A key prediction of this hypothesis would be that HONWEM optosilencing should not affect task motor execution in the absence of force field perturbation, where errors, and thus motor command updating by the model, are minor. To test whether this prediction was correct, we repeated the optosilencing experiments without the force field, and indeed found that neither the pull angle (Fig. 6E; two-tailed Mann–Whitney test,  $U_{(N_{\text{Control}} = 11, N_{\text{Optosilenced}} = 13)} = 64$ ,  $ns = p > 0.05$ ,  $n = 7$  mice), nor pull trajectory (Fig. 6F; two-tailed Mann–Whitney test,  $U_{(N_{\text{Control}} = 11, N_{\text{Optosilenced}} = 13)} = 53$ ,  $ns = p > 0.05$ ,  $n = 7$  mice) were significantly affected.

## Discussion

Our data show for the first time that mice are capable of performing a skilled forelimb task requiring motor adaptation to complex velocity-dependent force field perturbations, as previously observed in humans and nonhuman primates (Shadmehr and Mussa-Ivaldi, 1994; Perich et al., 2018). The robotic technology and experimental protocol described here can thus be used to study human-level motor control in a mouse model, allowing the dissection of underlying neural influences. We used this technology to explore a long-standing question: what is the role of endogenous activity of HONs, a type of neuron conserved between mice and humans and known to be an anatomic part of the motor system, in precisely defined elements of motor adaptation? At the correlational level, our results demonstrate that temporally-distinct endogenous waves of HON activity are associated with forelimb movements, and increase in amplitude during forelimb motor adaptation. At the causal level, we found that temporally-restricted optosilencing of these HONWEMs impaired sensory error-based adaptation. Crucially, HONWEM optosilencing affected neither the metrics of motivation such as reaction times and trial execution rates, nor forelimb motor outputs in tasks not requiring motor adaptation, indicating that the HON signals identified and studied were distinct from signals governing general motivation or muscle tone. Together, these results suggest that the endogenous HON signals facilitate motor adaptation during skilled forelimb movements.

### Role of endogenous HON activity in motor adaptation

The hypothalamus has been theorized to act as a key regulatory module in mammalian motor control for at least 50 years (Wayner, 1970; Arber and Costa, 2018). However, the function of specific hypothalamic cell types in defined aspects of acquired, adaptable movement patterns remained largely unknown. Our neural recordings and behavioral observations now provide clear evidence that HONs are endogenously active during certain skilled movements and contribute to forelimb motor adaptation. In the context of motor control, prior studies only looked at HONs from anatomic or reduced (in vitro) physiological perspectives, or looked at the effect of HON disruption on innate behavior (running, eating) without distinguishing motivation and motor components. Our new experimental approach, combining robotics with neural recordings and control, enabled us to separate sensory-error based motor control from reward and motivation metrics. This revealed that HON signals associated with forelimb movements impact sensory prediction error-based

motor adaptation, but not reward-based learning or motivation to do the task (Figs. 3, 4).

Thus, the lateral hypothalamus and HONs can be added to the growing list of brain regions involved in motor adaptation. It is noteworthy that brain regions implicated in motor adaptation in previous literature are innervated by HONs, specifically the cerebellum, cortical areas, and midbrain and striatal circuits (Peyron et al., 1998; Cutler et al., 1999; Nambu et al., 1999; Date et al., 2000; Korotkova et al., 2002; Bayer et al., 2004; Biswabharati et al., 2018). This suggests that HONs may implement motor adaptation both directly (via spinal cord projections; van den Pol, 1999; Yamuy et al., 2004) and/or indirectly by providing a modulatory influence to adaptation-implicated cortical and midbrain areas. Specifically, direct HON modulation of somatosensory cortex (Bayer et al., 2004) may be of particular importance, since the latter region was recently found to be an essential mediator of forelimb adaptation of a very similar type to that studied here (Mathis et al., 2017). Alternatively or in addition, HONs might indirectly assist forelimb motor adaptation through their modulation of locomotor and vestibular processes (Zhang et al., 2011; Karnani et al., 2020) that may contribute to overall postural adjustment that may help the new movement pattern to emerge. The relative functional importance of HON action on these different brain regions and processes in the context of motor control is a key subject for future work, for which our study provides an appropriate new mouse model.

Our conclusions about the role of endogenous HON activity in forelimb motor adaptation are based on similar experimental criteria performed in human studies. Velocity-dependent force fields allow for the perturbation to be dependent on the dynamics of the task, rather than applied at a fixed, arbitrary position, meaning that the internal model of task dynamics has to be remapped. Additionally, velocity-dependent force fields allow the same amount of force to be applied between long and short duration trials, i.e., total force is independent of time, whereas, with constant fields, the total force applied is dependent on the time spent in the field. Such velocity-dependent forcefields can only be implemented with advanced robotic technologies, which is a key methodological contribution of our work and advances the experimental paradigms applied in previous studies that typically rely on a fixed force field based on magnets (Mathis et al., 2017).

We found that mice follow broadly similar strategies to humans when their forelimbs adapt to perpendicular velocity-dependent force fields. They optimize for a strategy of overcompensation to the force field, rather than passive within-trial sensory feedback (Figs. 1G, 6B), suggesting sensorimotor remapping of the internal model. Our optosilenced data and sensory prediction error-based model suggests that HONs play an integral part in this remapping, and cannot be described by other features such as strength (Fig. 6A) or the complete sensory inability to perceive an external force (Fig. 6B).

Crucially, we also measured HON activity across trials where motor adaptation occurred (Fig. 2), revealing two unexpected features: activity waves associated with initiation and execution of skilled forelimb movements (Fig. 2B,C), and the increase in the amplitude of these waves across adaptation (Fig. 2D–F). This evolution of endogenous HON activity during the predictive/feedforward phase of each trial, i.e., before the mice start pulling and feel the force field, is consistent with a role of this HON activity in shaping predictive steering during motor adaptation (Fig. 6C). While our proposal about the role of HONs in emission

of new motor commands seems a plausible explanation given our data, at present we cannot completely rule out other explanations such as changes in muscle coordination, although the lack of effect of HON silencing in motor tasks not involving adaptation makes this unlikely (Fig. 6E,F). Internal model updating is likely to be a complex process involving multiple computational subprocess and brain areas such as the cerebellum (Wolpert et al., 1998), and it will be important to address how HONs interact with these areas in future work.

### Relation to previous studies of HONs in the context of arousal, motivation, and movement

Until now, HONs have been studied largely in the context of arousal and motivation. These studies revealed that loss of HONs in humans or mice makes wakefulness and muscle tone unstable, clinically known as narcolepsy with cataplexy, and orexin receptor antagonists stop rodents from working for reward (Nishino et al., 2000; Peyron et al., 2000; Thannickal et al., 2000; Hara et al., 2001; Boutrel et al., 2005; Harris et al., 2005; de Lecea et al., 2006; Harris and Aston-Jones, 2006; Mahler et al., 2014; Bassetti et al., 2019). Based on these previous conceptualizations of the endogenous role of HONs in goal-directed behavior, we expected HON optosilencing to reduce task execution rates. It was therefore surprising that HON optosilencing in our experiments affected neither motivation metrics such as trial rate, reward rate and reaction times, nor within-trial metrics such as movement velocity, arguing against major effects on muscle tone (Figs. 4C, 6A). However, we do not think our data contradict or dispute the earlier findings on the role of HONs in arousal and reward. Our study is fundamentally different because it targeted temporally and behaviorally defined HON signals (HONWEMs), whereas previous studies generally inhibited all HON signals for a prolonged time. It is not straightforward to draw parallels between these chronic effects of HON silencing, and acute effects studied here. Our results support the view that “motor” HON signals identified here are specifically involved in motor adaptation. This does not rule out that HON signals occurring at different times control arousal, reward, or muscle tone as previously proposed (Kiyashchenko et al., 2001; Milevskiy et al., 2002, 2005; Mochizuki et al., 2004; Lee et al., 2005; Mahler et al., 2014; Sakurai, 2014). Investigating candidate mechanisms that enable this HON “multitasking,” such as distinct temporal activity patterns of HONs and/or functionally different HON subpopulations, is a key subject for future work, for which the robotic tool presented here provides a suitable method that allows motivational/arousal and motor aspects to be distinguished.

In conclusion, despite numerous studies of the mechanisms of motor adaptation, genetically-defined hypothalamic signals that regulate it remained largely elusive. Our new mouse model of forelimb motor adaptation, which is directly translated from robotic paradigms in human motor adaptation studies, opens new possibilities for dissecting the neural underpinnings of motor adaptation. Our identification of a noncanonical role of hypothalamic neurons in adaptation illustrates the usefulness of this model, and provides a new genetically-defined entry point for probing neural circuits and signals that implement motor adaptation.

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