Corticostriatal neurons in auditory cortex drive decisions during auditory discrimination

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The neural pathways by which information about the acoustic world reaches the auditory cortex are well characterized, but how auditory representations are transformed into motor commands is not known. Here we use a perceptual decision-making task in rats to study this transformation. We demonstrate the role of corticostriatal projection neurons in auditory decisions by manipulating the activity of these neurons in rats performing an auditory frequency-discrimination task. Targeted channelrhodopsin-2 (ChR2)1–2–mediated stimulation of corticostriatal neurons during the task biased decisions in the direction predicted by the frequency tuning of the stimulated neurons, whereas archaerhodopsin-3 (Arch)3–mediated inactivation biased decisions in the opposite direction. Striatal projections are widespread in cortex and may provide a general mechanism for the control of motor decisions by sensory cortex.

After reaching the cortex, auditory information is processed and relayed to a number of cortical and subcortical targets by distinct, largely non-overlapping populations of pyramidal neurons. These targets include parietal cortex, secondary auditory areas, inferior colliculus and striatum, regions that have distinct roles in perception and behaviour. How these outputs help to establish associations between sounds and motor responses is unknown.

We sought to test the hypothesis that the projection from the auditory cortex to the striatum carries acoustic information that drives behavioural choices during auditory discrimination. The striatum receives topographic inputs from throughout the cortex. Striatal regions that receive input from motor and prefrontal cortices have been implicated in a wide range of cognitive processes, including decision-making4, action selection5 and reinforcement learning6,7. Through downstream structures of the basal ganglia, the striatum influences the activity in the motor thalamus8 as well as superior colliculus9, a structure that has been implicated in driving behavioural choices in two-alternative choice tasks10. Plasticity of corticostriatal connections may enable them to encode the arbitrary stimulus–response associations that are acquired in such tasks11.

In contrast, the function of striatal regions that receive direct input from sensory cortical areas is less well established. The striatum is one of the major long-range targets of the auditory cortex12. The auditory cortex projects to a specific region of the striatum13. This striatal region does not receive input from any other cortical area and contains neurons sensitive to auditory stimuli14–15. We set out to test whether the auditory corticostriatal projection drives choices during auditory discrimination.

We first developed a novel auditory discrimination task, inspired by experiments in primate middle temporal area (area MT)16, that was designed to exploit the tonotopic organization of the auditory system17. We trained rats to discriminate low- and high-frequency ‘cloud-of-tones’ stimuli in a two-alternative choice task18 (Fig. 1a). On each trial the stimulus consisted of a train of short overlapping pure tones distributed over a three-octave range (5–40 kHz). Subjects were required to choose between a left and a right reward port depending on whether the stimulus contained mostly low-frequency (5–10 kHz) or high-frequency (20–40 kHz) tones (Fig. 1b). Rats were free to withdraw and report their choice at any time after the onset of the stimulus. Rats readily learned this task. Performance varied smoothly with stimulus difficulty, and approached 100% on the easiest stimuli (Fig. 1c).

We used tetrode recordings to characterize the activity of individual neurons in the auditory cortex and the auditory striatum while rats performed the task. Auditory striatal neurons have been characterized previously in anaesthetized and passively listening animals14,15 but not during behaviour. The firing rates of neurons in both auditory cortex and striatum varied monotonically with the stimulus (Supplementary Fig. 1a, b, e, f). ‘Ideal observer’ analysis showed that stimulus selectivity of cortical and striatal neurons was similar (Supplementary Fig. 1c, d, g, h). Although individual neurons rarely matched the performance of the rats, many neurons in both areas could discriminate the stimulus above chance levels (Supplementary Fig. 1d, h). These observations indicate that neurons in the striatum preserve the information available to perform the cloud-of-tones task.

We then tested whether manipulating cortical activity with ChR2 (refs 1, 2) could affect subjects’ choices in the task. We proposed that activation of auditory cortical neurons in different regions of the tonotopic map would result in choice biases, the directions of which would depend on the frequency–response association that the rat had been trained to make. We implanted arrays of optical fibres coupled to tetrodes (Supplementary Fig. 2), which enabled us to characterize the frequency preference of neurons near the fibre tip. We activated ChR2-expressing auditory cortex neurons on a subset of trials, and...
compared performance on stimulated trials to that on control trials without light activation. To minimize behavioural adaptation to photostimulation, we limited the number of stimulation trials to 25% of the total trials, and rewarded the animal as in control trials, according to the frequency content of the stimulus. To quantify the effects of photostimulation on subjects’ behaviour, we fit a logistic regression model to the subjects’ choices (see Methods), estimating what change to the auditory stimulus would produce a behavioural effect equivalent to that of photostimulation.

We first expressed ChR2 non-specifically in the primary auditory cortex using adeno-associated virus (AAV). ChR2 expression was distributed throughout layers II to V and was present, although to a lesser extent, in layer VI (Supplementary Fig. 3a, b). In some sessions, activation of ChR2-expressing neurons induced substantial biases (Supplementary Fig. 3c). However, contrary to our expectations the direction of the bias was not predicted by the tuning of the neurons near the fibre (Supplementary Fig. 3f). Moreover, this non-specific activation not only biased subjects’ choices but also interfered with their ability to perform the task, as measured by the slope of the psychometric curve (Supplementary Fig. 3d, e).

The lack of correspondence between the direction of stimulation-evoked choice biases and frequency tuning was surprising and has several possible explanations. First, these choice biases may result from activation of intracortical projection neurons from other frequency bands of the auditory cortex. Second, they may arise from preferential activation of local interneurons in response to synchronous activation of a large number of cortical cells. Third, these biases may be a consequence of stimulation of competing output pathways, projecting to target areas with different functional roles. Consistent with this third possibility, stimulation resulted in predominantly contralateral biases (36 out of 57 sites, $P = 0.015$, Supplementary Fig. 3g, h), suggesting the activation of a lateralized motor pathway. These results suggested that the auditory cortex plays a role in the task, but that the method of non-specific activation of diverse neuronal populations used in these experiments does not provide sufficient experimental control over the activity of the projection neurons driving choices in this behaviour. We therefore proposed that targeted stimulation of corticostriatal neurons might produce more systematic effects.

We used two complementary strategies to test the role of the neuronal subpopulation in auditory cortex that projects to the striatum. In the first strategy, we targeted ChR2 specifically to corticostriatal neurons using a herpes simplex virus-1 (HSV-1) engineered to express Cre recombinase19–21 (Fig. 2a). This virus, when injected into the auditory striatum, was transported retrogradely along the axons and drove Cre expression in corticostriatal neurons. We then injected a Cre-dependent AAV driving expression of ChR2 into the auditory cortex, resulting in expression of ChR2 only in neurons infected by both the HSV-1–Cre and the AAV-ChR2. Most (84%) of ChR2-expressing neurons were located in layer V (Fig. 2c, d), consistent with previous results22.

Pulses of blue light delivered through fibres in the cortex reliably drove action potentials in a small population of presumed corticostriatal neurons (Fig. 2b; only 4 of 201 cells responded in more than 50% of trials). Across sites, stimulation consistently biased subjects’ choices towards the choice port associated with the preferred frequency of the stimulation site (Fig. 2e, f). The biases were not significant in individual sessions ($P < 0.05$ in only 1 out of 33 sessions), partly because the number of stimulated trials per session was typically small (70 ± 24 (± s.d.) trials). In contrast to non-specific stimulation, contralateral and ipsilateral biases were observed with similar frequency (12 out of 33 contralateral sites, $P = 0.31$, signed-rank test) and subjects’ psychophysical performance was not impaired (Supplementary Fig. 4a). Choice biases were not observed in un.injected controls (Fig. 2g and Supplementary Fig. 5a), indicating that they were driven by the ChR2-mediated activation of corticostriatal neurons and not non-specific effects of light delivery. Thus, activation of corticostriatal neurons biased behaviour in a manner predicted by their frequency tuning.

The behavioural effects of photostimulation could arise either directly through excitation of striatal neurons by their cortical inputs, or indirectly through excitation of other output pathways of the auditory cortex through recurrent connections. Excitation of corticostriatal neurons resulted in general suppression of local cortical activity (Supplementary Fig. 6), favouring the hypothesis that behavioural effects of photostimulation are mediated by long-range rather than local outputs of these neurons.
To test this hypothesis directly, we used a second strategy. This strategy did not rely on cortical stimulation and therefore enabled us to test whether stimulation of corticostriatal neurons could bias subjects’ choices when recurrent cortical activity was blocked. We stimulated the axons of corticostriatal neurons in the striatum while inhibiting the recurrent cortical excitation pharmacologically (Fig. 2h). To define the frequency tuning of stimulated fibres, we exploited the topography of corticostriatal projections23. We confirmed the topographic organization of the striatal projection of the auditory cortex in rats through retrograde (Supplementary Fig. 7) tracing. Owing to this topography, focal light stimulation in the striatum excites corticostriatal axons arising from a restricted region of the tonotopic map. We used striatal multi-unit activity to characterize the frequency preference of the striatal stimulation site.

Stimulation of corticostriatal axons biased subjects’ choices towards the choice port associated with the preferred frequency of the stimulation site (Fig. 2g and Supplementary Fig. 5b). Stimulation also had a modest effect on the subjects’ performance at sites with large choice biases, possibly owing to a ceiling effect (Supplementary Fig. 4b, c). Thus, both direct activation of corticostriatal somata in the cortex, as well as antidromic activation of corticostriatal terminals in the striatum, biased the rats’ choices in a manner predicted from the frequency tuning of the activated neurons.

We next tested whether stimulation of the axons of corticostriatal neurons could bias subjects’ choices in the absence of cortical recurrent activity. We selected striatal sites whose stimulation produced significant biases (P < 0.05) in subjects’ choices and repeated stimulation after pharmacologically inactivating the ipsilateral auditory cortex. Cortical inactivation (2% lidocaine, n = 2 sites; 125 μM tetrodotoxin, n = 5 sites) blocked antidromic light-evoked local field potentials (Fig. 2i). Photostimulation of corticostriatal axons still biased choices in the absence of cortical activity (P = 0.016, signed-rank test, Supplementary Figs 8 and 9), demonstrating that these biases are mediated directly by long-range outputs of corticostriatal neurons.

We next examined the effects of stimulation of corticostriatal neurons on rats’ response times. Although individual rats varied in how they prioritized response speed versus response accuracy (Supplementary Fig. 10a–f), most rats used in the stimulation and inactivation experiments showed a small but significant increase (in the order of 20 to 50 ms) in response time when challenging trials, consistent with this reported previously in rats18. We quantified the shift of the chronometric curve produced by stimulation of corticostriatal neurons (Supplementary Fig. 10g, h). Across sessions, stimulation shifted chronometric curves, mimicking the effect of adding acoustic evidence favouring the choice associated with the preferred frequency of the stimulation site (Supplementary Fig. 10i, j).

So far, our results establish that activation of the corticostriatal pathway is sufficient to bias subjects’ choices during an auditory discrimination task, but do not establish whether corticostriatal activity contributes to the formation of decisions under normal conditions, in the absence of ChR2 activation. If corticostriatal spikes are important under normal conditions, then suppressing these spikes during the task should lead to an ‘anti-bias’; that is, a bias in the direction opposite to that induced by ChR2 activation. To test this hypothesis, we targeted the expression of the light-activated proton pump Arch1 to corticostriatal neurons using the HSV-1-based approach described above (Fig. 3a). Rather than attempting to inactivate all corticostriatal cells—a technically difficult feat whose interpretation may be obscured by the compensatory plasticity common to lesion studies—we sought instead to silence corticostriatal neurons within a restricted region of the tonotopic map.

Pulses of green light in Arch-expressing animals inhibited spiking of putative corticostriatal neurons (Fig. 3b). As predicted, inactivation of corticostriatal neurons biased subjects’ choices away from the reward port associated with the frequency band of the inactivation site (Fig. 3c) but did not affect subjects’ sensitivity quantified as the slope of the psychometric curve (Supplementary Fig. 4d). A similar trend was reflected in the subjects’ chronometric functions but did not reach significance (Supplementary Fig. 11k, l). Pulses of green light did not affect the behaviour of uninjected control animals (Supplementary Fig. 5c). These results indicate that corticostriatal neurons in the auditory cortex transmit signals used by rats to make decisions driven by sounds.

These experiments provided us with an opportunity to quantify the contribution of the output of single corticostriatal cells to behaviour. Earlier studies showed that subjects can be trained to detect the artificial stimulation of as few as 6 to 197 neurons24 or even a single neuron25. Our experiments allow us to estimate the contribution of single neurons to behaviour during normal perception.

We tagged Arch with green fluorescent protein (Arch–GFP) to estimate the number of neurons silenced in each experiment (Fig. 4b). Using in vivo recordings of putative corticostriatal cells, we estimated that the efficiency of inactivation decayed with a space constant of approximately 564 μm (Fig. 4a and Supplementary Fig. 11). We quantified the number of Arch-expressing neurons within a radius of 1 mm, or approximately two space constants, from the inactivation fibre for each session. The magnitude of choice biases depended on the number of corticostriatal neurons expressing Arch near the fibre (Fig. 4c). Robust choice biases were observed for sites with 1,040 to 2,444 Arch-expressing neurons within 1 mm of the fibre, comprising 0.35 to 0.83% of neurons in that region.
volume. Our observation that inactivation of this minute fraction of cells targeted to corticostriatal neurons could affect behaviour suggests a privileged role of these cells in auditory discrimination.

The size of the neuronal pool accounting for behavioural performance in psychophysical tasks is much smaller than the number neurons which carry signals relevant to the task. The redundancy arising from correlated neuronal firing may restrict the effective size of the pool, limiting psychophysical performance. Our results suggest that the finite bandwidth provided by a small number of projection neurons that drive decisions constitutes an additional constraint limiting the effective pool size and therefore psychophysical performance.

We have shown that the striatal projection of the auditory cortex conveys signals that drive behavioural choices during performance of an auditory discrimination task. Although our results do not exclude the participation of other parallel pathways, the ubiquity of corticostriatal projections in cortex may provide a general mechanism for control of motor decisions by sensory context.

**METHODS SUMMARY**

Male Long Evans rats (Taconic Farms) were trained in a two-alternative choice discrimination task. Animal procedures were approved by the Cold Spring Harbour Laboratory Animal Care and Use Committee and carried out in accordance with National Institutes of Health standards.

Full Methods and any associated references are available in the online version of the paper.

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FROM THE TARGET OCTAVE WITH A PROBABILITY OF (1 − b1) AND FREELY AVAILABLE FOR 15 TO 30 MIN AFTER THE END OF EACH BEHAVIOURAL SESSION.

ANIMAL SUBJECTS. Animal procedures were approved by the Cold Spring Harbour Laboratory Animal Care and Use Committee and carried out in accordance with National Institutes of Health standards. Male Long Evans rats (Taconic Farms) were housed with free access to food, but were water restricted after the start of behavioural training. Water was available during task performance (24 μl for each correct trial) and freely available for 15 to 30 min after the end of each behavioural session and for at least 1 h on days when behavioural sessions were not conducted.

VIRAL INJECTION. Three- to five-week-old rats were anaesthetized with a mixture of ketamine (60 mg kg−1 of body weight) and medetomidine (0.24 mg kg−1) and placed in a stereotaxic apparatus. To target the auditory cortex, part of the temporoparietal bone was resected to expose the temporoparietal suture, which was used as a landmark to target injections. For optogenetic experiments, four injections were made unilaterally spanning primary auditory cortex at 0.5, 1.5, 2.5 and 3.5 mm from the rostral edge of parietal bone and 1.2 mm from its ventral edge. A small craniotomy was made for each injection and a glass micropipette was inserted perpendicular to the surface of the brain. Two injections were made at the depths of 400 and 800 μm, expelling approximately 250 nl of virus at each depth. To target the auditory striatum, two small craniotomies were made 2.0 and 2.5 mm caudal of Bregma and 4.5 mm lateral of the midline. Injections were made at depths between 3.5 and 6 mm, 0.5 mm between injection sites, approximately 100 nl per site. Injections were carried out by delivering brief pulses of pressure using Picospiglet II (Parker), each pulse delivering approximately 2 nl at 0.2 Hz. Rats were monitored during their recovery from surgery and returned to group housing.

BEHAVIOURAL TRAINING. The cloud-of-tones stimulus consisted of stream of 30-ms overlapping pure tones presented at 100 Hz (that is, with 10 ms between tone onsets). Eighteen possible tone frequencies were logarithmically spaced between 5 and 40 kHz, a range in which rats’ hearing thresholds are low and relatively constant. For each trial either the low (5 to 10 kHz) or the high (20 to 40 kHz) octave was selected as the target octave. Stimulus strength r determined the difference in the rate of high and low octave tones in the stimulus. Tones were drawn from the target octave with a probability of (1 + 2r/100)/3. The stream of tones continued until the rat withdrew from the centre port.

After reaching a weight of 200 to 250 g, rats were placed on a water deprivation schedule and behavioural training commenced. The rats were placed in a sound-proof behavioural chamber and presented with three choice ports. The rats were trained to first poke into the centre port, wait for the onset of the auditory stimulus and select one of the other two ports to receive a water reward (24 μl). Rats were trained to carry out this procedure using the following procedure. During the first phase of training, water was delivered at the correct choice port as soon as the stimulus was played. The duration of the pre-stimulus delay, during which the rat was required to remain in the centre port, was drawn from an exponential distribution whose mean was gradually increased from 0.05 to 0.3 s. The next phase of training required the rat to poke at the correct choice port to trigger water delivery. However, the rat was allowed to correct his choice if it made a mistake. When rats had learned to perform the discrimination so that they consistently scored higher than expected by chance, they were required to make the correct choice on the first attempt. Error trials were punished with a 4-s timeout (2 s during recording sessions). Initially, rats were trained to discriminate tone-cloud stimuli composed of tones either entirely in the high octave or entirely in the low octave (τ = 100) and were gradually introduced to increasingly difficult stimuli.

Sound intensity of individual tones was constant during each trial. To discourage subjects from using loudness differences in discrimination, tone intensity was randomly selected on each trial from a uniform distribution 45 to 75 dB (SPL) during training. During manipulation and recording sessions, sound intensity was kept constant at 60 dB.

ELECTROPHYSIOLOGY AND OPTOGENETICS. Custom-built optical fibre and tetrode arrays were assembled in-house. Each array carried 6 multimodal optical fibres 62.5 μm in diameter with a 50-μm core. The fibre tips were sharpened using a diamond wheel to improve tissue penetration and increase the angle of the light exit cone. Each fibre was glued to a tetrode and the tetrode tip was cut to terminate within approximately 100 μm of the fibre tip. The fibre and tetrode assemblies were mounted on individually movable microdrives. The tetrodes were gold-plated to an impedance of 300 kΩ at 1 kHz and the tetrode and fibre tips were coated with Dif fluorescent dye (Life Technologies) to assist with the identification of fibre tracks in brain tissue.

To implant the fibre and tetrode array, rats were anaesthetized with a mixture of ketamine (40 mg kg−1 of body weight) and medetomidine (0.16 mg kg−1) and placed in a stereotaxic apparatus. A craniotomy was made over the target area (for auditory cortex, 3.5 to 6.0 mm caudal of Bregma and 6.5 to 7.0 mm lateral from the midline; for auditory striatum, 2.5 to 3.5 mm caudal of Bregma and 4.0 to 5.5 mm lateral from the midline). All rats, with the exception of one Arch-expressing and one uninjected control animal, were implanted in the left hemisphere. The dura was removed and the implant was placed over the target area and fixed in place with dental acrylic. The tetrodes were then lowered until the first action potentials were encountered.

To characterize the frequency tuning of stimulation and inactivation sites, the rats were placed in a soundproof chamber and pure tones were played in free field at approximately 0.5 Hz. Tone frequencies spanned from 1 to 64 kHz and were played in a random order at 30, 50 or 70 dB-SPL. Only sites that significantly responded to sounds (P < 0.01, signed-rank test comparing firing rate 5 to 55 ms after sound onset 10 to 50 ms preceding sound onset) were included in the analysis of stimulation and inactivation experiments. To determine the preferred frequency, firing rates in the window from 5 to 55 ms after sound onset were computed for each frequency at 70 dB. The resulting tuning curve was smoothed with a half-octave sliding window. The peak of the smoothed tuning curve was selected as the preferred frequency. Sites tuned to frequencies more than one octave outside the range used in the task (below 2.5 kHz) were excluded from analysis.

For optogenetic manipulations, laser light was coupled into a fibre-pair Collimator (Thor Labs). Laser power was adjusted to produce the desired output at the end of the patch cord. A single implanted fibre was selected for manipulation and coupled to the patch cord. For ChR2 activation, 473-nm laser light (10 mW) was delivered in 1-ms pulses (except at nine sites) for cortical stimulation. AAV-CAGGS-FLEX-ChR2-tdTomato virus was used for corticostriatal stimulation. AAV-FLEX-Arch–GFP construct was targeted to primary auditory cortex. AAV-CAGGS-Venus plasmid was used for non-specific cortical stimulation. AAV-CAGGS-FLEX-ChR2-tdTomato virus was used for targeted stimulation of corticostriatal neurons. AAV-FLEX–Arch–GFP construct was generated by subcloning Arch–GFP from an AAV-CAG–Arch–GFP plasmid into AAV-FLEX backbone from AAV-EF1α-FLEX-Chr2–YFP. The plasmid DNA was prepared using standard Maxiprep protocols (Qiagen). AAV serotype 2/9 was packaged by the University of North Carolina viral core at a titre of 1 to 2 × 1010 particles per ml. HSV–mCherry–IRES–GFP construct (for targeting Arch expression) and HSV–IRES–2A-Venus construct (for targeting ChR2 expression) were packaged at titres of 2.4 × 1010 and 3 × 1010 transducing units per ml.

Pharmacological inactivation. Two PEEK tubing cannulas (Plastics One) were implanted into the auditory cortex with a separation of approximately 1 mm. Six stereotrodes were implanted alongside the cannulas, spanning 3 to 4 mm of the auditory cortex, to confirm the efficiency of inactivation. One hour before the start of the behavioural session, the animal was briefly anaesthetized with 2% isoflurane and 0.4 μl of drug (during inactivation sessions) or 9 g l−1 NaCl (during control sessions) was injected at the rate of 0.08 μl min−1 in both cannulas.

ANALYSIS OF BEHAVIOURAL DATA. Although each trial had a target frequency distribution, which determined which choice port was to be rewarded, as rats sampled the stimulus for a finite period of time, the frequency distribution they experienced might have been substantially different from the target. Therefore, we determined the rate of presentation of high and low tones (fhigh and flow), that is, the number of tones divided by the reaction time, that the rats actually heard on each trial. To quantify rats’ behaviour, psychometric curves were fit with a logistic regression model:

\[
\ln \frac{p}{1-p} = \beta_0 + \beta_1 (f_{\text{high}} - f_{\text{low}})
\]

(1)

where \( p \) is the fraction of choices towards the port associated with high frequencies. Parameters \( \beta_0 \) and \( \beta_1 \) measure the bias and slope of the psychometric curve, respectively. To quantify the effects of stimulation or inactivation, we extended the model to incorporate the effects of these manipulations:

\[
\ln \frac{p}{1-p} = \beta_0 + \beta_1 (f_{\text{high}} - f_{\text{low}}) + S + \beta_2 S (f_{\text{high}} - f_{\text{low}})
\]

where \( S \) is 1 on manipulation trials and 0 on control trials. The choice bias evoked by the manipulation is \( \beta_2 (f_{\text{ch}} + f_{\text{r}}) \) tones per s. In experiments in which ChR2 or Arch expression was specifically targeted to corticostriatal neurons, the interaction term \( \beta_2 \) was not significantly different from 0 and was omitted in estimates of choice bias.

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The light-evoked choice bias is then \( \beta_1 / \beta_0 \) tones per s. Confidence intervals for \( \beta_1 \) and \( \beta_0 \) estimates were derived through bootstrap resampling. A small number of sites (2 out of 59 using non-specific cortical stimulation; 4 out of 56 using striatal axonal stimulation) produced extreme choice biases (greater than 200 tones per s) outside the stimulus range tested in the task and were excluded from regression analysis. Including these sites did not alter the conclusion that striatal axonal stimulation tended to bias subjects equal to the discrimination threshold were scored as reporting the preferred frequency of the neuron. The neuronal choices were then fit with a logistic regression model (see equation (1)).

To calculate peristimulus time histograms (PSTHs), neuronal firing rates were smoothed with a causal half-Gaussian kernel (\( \sigma = 5 \) ms). Confidence intervals were derived through bootstrap resampling. For each Arch-expressing corticostriatal neuron that we encountered, we delivered light at different fibres along the array to estimate the maximum distance at which light delivery could reduce the neuron’s firing by 50%. We took this distance to be the mean of the distances to the furthest fibre for which inactivation was greater than 50% and the closest fibre for which inactivation was less than 50%. The likelihood of inactivation at a given distance was estimated as the fraction of neurons inactivated at that point.

**Histology and cell-count analysis.** At the end of the experiment, rats were deeply anaesthetised with ketamine and medetomidine. Small electrically lesions were made by passing 30 \( \mu A \) direct cathodal current through each tetrode for approximately 10 s, marking the final position of the tetrode tip. The rats were then perfused with 4% paraformaldehyde (PFA), their brains were extracted and post-fixed in 4% PFA overnight. The brains were cut into 100-\( \mu m \) sections and mounted using Vectashield (Vector Laboratories) for confocal microscopy. To ensure that HSV-mediated labelling was confined to corticostriatal neurons, we verified that viral expression was absent in adjacent brain structures.

To quantify the depth distribution of opsin expression, we measured the distance from each fluorescent cell soma to the pia as a fraction of total cortical thickness. To estimate the number of neurons affected by optogenetic manipulations, confocal stacks 50 \( \mu m \) in depth were acquired from alternate sections. The sections were registered using rigid registration maximizing the cross-correlation of fluorescence images of adjacent sections. The locations of ChR2- or Arch-expressing neurons were identified manually. Fibre tracks were identified with the help of electrolytic lesions and DiI labelling. Using the locations of the ends of the tracks, we estimated that processing resulted in approximately 10% shrinkage of the tissue. We estimated the location of the fibre tip during each inactivation session and counted the number of expressing neurons within 1 mm from the fibre at 35 sites illuminated with 5 mW light. At 6 sites illuminated at 2.5 mW we reduced the radius according to square root of the power to 0.7 mm. Excluding these sites from the analysis did not affect our conclusions (\( P = 0.036, \) linear regression of cell count and choice bias). As we only identified cells within a 50-\( \mu m \) stack every 200 \( \mu m \), our estimate of the total number of manipulated cells is four times this count.

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