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Perturbations in the Activity of Cholinergic Interneurons in the Dorsomedial Striatum Impairs the Update of Actions to an Instrumental Contingency Change

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Abstract—The striatal cholinergic system is key in detecting changes in instrumental contingencies. While recent evidence supports this vision, cell type-specific online control on the activity of the cholinergic striatal neurons is necessary to empirically test it. In this study, we performed optogenetic manipulations of the activity of striatal cholinergic interneurons (CINs) to evaluate their contribution to the updating of a previously learned instrumental contingency. By modulating the activity of CINs, we identified that the inhibition of CINs impairs the update of actions to a contingency change. Remarkably, a manipulation that perturbs the activity of CINs, rather than inhibiting them also impaired the encoding of the change in contingency. These results emphasize that beyond an increase in the activity of CINs, the intact activity of these cells is required for the identification of an instrumental contingency change.

Key words: striatal cholinergic interneurons, optogenetic manipulations, contingency degradation, behavioral flexibility.

INTRODUCTION

The identification of changes in contingencies allows animals to update memories and adapt their behavior. The prefrontal cortex sub-circuits innervating the striatum, mainly the dorsomedial striatum (DMS), have been shown to be critical for the update of behavior to changes in instrumental contingencies (Balleine and Dickinson, 1998; Yin et al., 2005). Later on during the investigation of the cellular mechanisms behind the contribution of the DMS to the detection of changes in contingencies, several studies have documented that the activity of putative striatal cholinergic interneurons (CINs) correlate or is necessary for subjects to identify contingency modifications between an action and its outcome (Aosaki et al., 1994; Atallah et al., 2014; Aoki et al., 2015; Bradfield et al., 2013; Matamales et al., 2016; Okada et al., 2014; Ragozzino, 2003). Specifically, the first study that recorded the activity of putative striatal CINs (tonically active neurons; TANs) in primates, documented that during the acquisition of sensory associations these TANs become responsive to a stimuli paired with a reinforcer that induced the conditioned behavior, showing a pattern of depolarization-pause-depolarization spiking lasting ≈300–400 ms, (Aosaki et al., 1994). Three years later another study recording TANs in monkeys suggested that both, the stimuli predicting the reinforcer, and the reinforce itself, could evoke a pause in the spiking of TANs of around ≈300 ms (Apicella et al., 1997). A high temporal modulation of putative CIN's, during the update of contingencies, has been also observed in rodents, suggesting that CINs present an increase in spiking immediately after receiving an uncertain reinforcer (Atallah et al., 2014). In parallel to the fast temporal electrophysiological recordings of putative CINs in primates and rodents, a fast increase and return to baseline levels in acetylcholine (Ach) in the DMS of rats during the improvement of reversal learning of a spatial task was documented (measuring Ach in six blocks, 6 min interval, measured by microdialysis), observing that the maximum peak of Ach was not on reaching the best performance but on the blocks where animals seem to present a shift in strategy (Ragozzino, 2003), suggesting that the striatum, and in particular the CINs, were required for behavioral flexibility. Afterward, lesion and pharmacological studies strongly implicated a thalamo-DMS circuit specifically modulating the activity of CINs for the proper performance of behavioral flexibility

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Abbreviations: DMS, dorsomedial striatum; ChAT, choline acetyltransferase; Cre, enzyme cre-recombinase; CINs, cholinergic interneurons; ROC, receiver operating characteristic curve; CDF, cumulative distribution function; ISI, interspike interval; A1→, O1, Action 1 to Outcome 1; A2→, O2, Action 2 to Outcome 2; RR, random ratio.

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in spatial tasks (Brown et al., 2010) or during the change of contingencies in instrumental learning (Bradfield et al., 2013). However, and despite the strong implication of striatal CINs in mediating behavioral flexibility, only when transgenic mice that specifically labeled cholinergic cells became available (ChAT-Cre mice; Gong et al., 2007) could the CINs be specifically ablated to evaluate this possibility. The first study performing ablations of striatal CINs reported that their absence caused enhanced flexibility in a spatial discrimination task (Okada et al., 2014). Of particular interest for this study, a second study documented that CINs in the DMS determine the strategy set shifting (Aoki et al., 2015), suggesting that they may be required to detect changes in instrumental contingencies (Matamales et al., 2016). In conclusion, based on the current literature there is a wide set of studies providing support to the hypothesis that the activity of CINs is required for the identification of changes in instrumental contingencies. However, an important deficiency of all these studies is that no study has specifically manipulated the activity of CINs during the updating of instrumental contingencies to test the feasibility of this hypothesis. Therefore, to evaluate the contribution of the activity of CINs to changes in instrumental contingencies, we performed optogenetic manipulations, which allow temporal, spatial and cell type specific perturbations of activity, during a behavioral task previously described to evaluate the abilities of mice to update the identification of a change in an instrumental contingency (Balleine and Dickinson, 1998; Bradfield et al., 2013; Matamales et al., 2016; Yin et al., 2005). A temporal inhibition of CINs around the performance of the action was tested. Remarkably, we observed that optogenetic inhibition, or a perturbation of the activity of DMS CINs that does not decrease the overall firing of CINs, impaired the identification of the change in the instrumental contingency, suggesting that beyond an increase in their activity, the intact activity of these cells is required for the update of actions to instrumental contingency changes.

EXPERIMENTAL PROCEDURES

Animals

All use of animals was approved by the Institutional Committee for the Care and Use of Laboratory Animals of Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (Protocol number FTA121-17) and the National Norm for the use of Animals (NOM-062-ZOO-1999). C57BL/6J wild type mice between 2 and 4 months old were used to standardize the behavioral task, and heterozygous ChAT-Cre mice of the same age (male and female; B6;129S6-Chat$^{tm2(cre)Lowl}$/J) (Jackson labs # 006410) (Gong et al., 2007) were used for optogenetic manipulation experiments. Additionally, a ChAT-Cre:Ai35 (Madisen et al., 2012) crossbred mouse was used to verify the specificity of the ChAT-Cre line. All animals were housed under a 12:12 h light/dark cycle. Food, standard rodent chow (LabDiet) and water (5 min daily access) were restricted in order to keep ~85% of basal weight during behavioral training.

Behavioral training

All training was conducted during the light period and based on previous protocols developed to evaluate the capability of rodents to detect the change in an instrumental contingency (Balleine and Dickinson, 1998; Bradfield et al., 2013; Corbit and Balleine, 2000; Matamales et al., 2016; Yin et al., 2005).

Pre-training. Animals were allowed to explore an operant chamber (Med Associates Inc) for two sessions (30 min each session; ~2 h delay between sessions) in one day. In session 1 a pellet (14 mg, Bio-Serv) was delivered randomly (every 30 s there was a 50% chance of delivery); in session 2, a drop (~14 μL) of 20% sucrose (SIGMA-Aldrich) was delivered instead.

Continuous reinforcement. Animals were trained for two sessions per day for three days. During each session only one of two levers, to the left or right side of the feeder, was presented. During session 1, the left lever was exposed. Each lever press (action A1) delivered the same reinforcer (outcome O1) throughout the session (either a pellet or a drop of sucrose). On session 2 the second lever was presented to the right of the feeder, and each lever press (action A2) delivered the other reinforcer (outcome O2). That is, animals that received pellets in session 1 now received a drop of sucrose and vice versa. Each session finished after 20 reinforcers or 30 minutes, whichever came first. The relation Action1-Outcome1 (A1 → O1) and Action2-Outcome2 (A2 → O2) was counterbalanced for all groups.

Random ratio training. The same animals were trained in a random ratio (RR) schedule for ten days. First, subjects were trained 2 days in RR5: for each session ~5 lever presses delivered an outcome. Afterward, subjects were trained in RR10 (~10 lever press = outcome) for 2 days and finally in RR20 (~20 lever press = outcome) for 6 days. Each session finished after 20 reinforcers or 30 min (Fig. 1A).

Devaluation test. To test the A1 → O1 and A2 → O2 association in RR training, animals were allowed *ad libitum* access to one of the two outcomes for 1.5 h. This was done to reduce the value of the satiated outcome in comparison to the other (Balleine and Dickinson, 1998). Immediately after, animals were placed in the conditioning chamber with both levers exposed, and lever presses were recorded for 10 min. The next day, a second devaluation test was performed using the opposite outcome. Groups were counterbalanced (Fig. 1B).

Contingency degradation training. Following the devaluation test, animals were trained for 5 days with a change in the instrumental contingency. During each session only one lever was presented either at the left or the right of the feeder. Session 1 was changed to degrade the action-outcome relationship: free reinforcers were delivered with a probability of 0.025 every second that the animal did not press the lever

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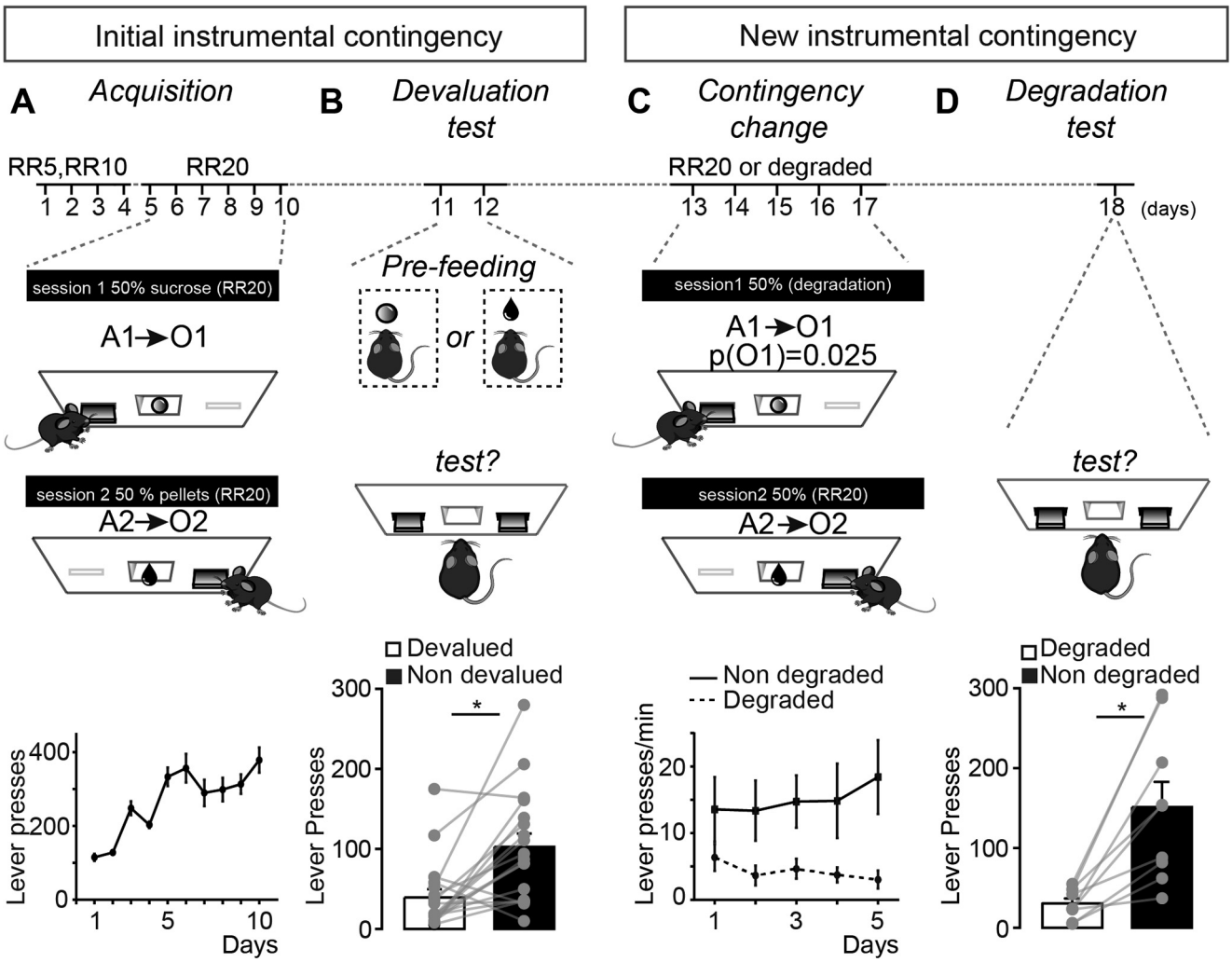

Fig. 1. Description of the behavioral training protocol to evaluate a change in instrumental contingency in mice. (**A**) Upper panels, from top to bottom: training days, representation of the two sessions a day of training for animals to acquire the two actions → two outcomes relationship. Note that in each session there is only one lever. Bottom panel, mean data of both sessions per day from nine wild type mice. (**B**) Upper panels, days in devaluation test, and representations of the procedure: before testing, each had a pre-feeding session on one of the reinforcers. Bottom panel, mean data from the two devaluation sessions of the same animals in **A** (18 sessions). (**C**) Upper panels, days and representations of the degradation training: one session was kept in random ratio 20 (RR20) and the other in contingency degradation (deg): [no press/s = 0.025(O1)]. Bottom panel, presses represented in rate for each session from the same animals in (**A**, **B**). (**D**) Upper panels, representation of the degradation test where unfed animals are exposed to the two levers. Bottom panel, data from the same animals in (**A**–**C**). *$p < 0.05$, Wilcoxon (paired) and Mann Whitney *U* tests (bars).

(this was termed the Degraded Session). Session 2 maintained the same contingency of RR20 schedule (termed the Non-Degraded Session). The order of the sessions (either Session 1 or Session 2) and the degraded outcome (either pellet or sucrose) was counterbalanced across animals for each group. All sessions degraded or non-degraded, finished after 20 outcomes or 30 min (Fig. 1C).

Degradation test. Finally, after degradation training, we tested the relationship between the non-degraded A1 → O1 and the A2 → O2 degraded conditions. To this end, unfed animals were introduced to the operant chamber with both levers presented. Lever presses were recorded for 10 minutes without delivery of any outcome (Fig. 1D).

Optogenetic manipulation of striatal CIN

Stereotaxic surgery. To transfect inhibitory opsins, ChAT-Cre mice were anesthetized with ∼1.5% isoflurane (in 1 liter/min oxygen) and placed in a stereotaxic apparatus. Using glass pipettes (Drummond Scientific), 500 nL (per hemisphere) of viral stock [AAV5-EF1a-DIO-eArch3.0-EYFP (Vector core, University of North Carolina, titer $\geq 1 \times 10^{13}$ vg/mL)] was delivered bilaterally into DMS (coordinates: AP −0.1, ML ±1.8 and DV −2.2); this region of the DMS have been previously shown to impair new goal-directed memories (Yin et al., 2005; Bradfield et al., 2013; Matamales et al., 2016). Injections were performed with a Nanoject-II (Drummond Scientific) programmed to deliver 4.6 nanoliters every 5 s (23 nL/s rate). Control animals

(ChAT-Cre:eYFP) were transfected with AAV1.EF1a.DIO.eYFP.WPRE [Vector core, University Pennsylvania (UPENN), titer $\geq 1 \times 10^{13}$ vg/mL]. After allowing 15 minutes for diffusion, a custom-made optic fiber [length: ~9 mm, diameter: 300 μm, NA: 0.39 (FT300EMT, Thorlabs) coupled to a stainless steel ferrule (SFCL340-1, Thorlabs)] was implanted into each hemisphere 100 μm above viral injection coordinates (Fig. 2C, E). Optical fibers were fixed with acrylic cement (Lang Dental Manufacturing). Three weeks were allowed for viral expression before optogenetic manipulation.

Optogenetic manipulation during behavior. The activity of transfected CINs was manipulated with a green laser (556 nm, CNI Laser), whose beam path was controlled by an acousto-optic modulator/shifter (MTS110-A3-VIS, Opto-Electronic) with a risetime of 1 μs, driven by a Fixed Frequency Driver (AA Opto-Electronics) and a pulse generator (Arduino cc) (Fig. 2D). Three photo-inhibition protocols were tested: (1) continuous light, (2) 20 ms laser on at 10 Hz (20 ms-10 Hz) and (3) 3 ms laser on at 10 Hz (3 ms–10 Hz) (Fig. 2E). Power intensity was calculated through predicted irradiance values using available data (https://web.stanford.edu/group/dlab/cgi-bin/graph/chart.php). For continuous inhibition, we used 15 mW while for the pulsed protocols 25 mW at the tip of the fibers was used. To investigate the effect that the CIN s activity manipulation has on the recognition of an instrumental contingency change online, all groups were subjected to optogenetic manipulations during the degradation sessions. A 4 cm by 4 cm region centered around one of the levers was defined through a camera-assisted custom movement detection software (written in C#), as the inhibition zone (Fig. 2D). Each time animals crossed this zone the software triggered the laser delivery to the implanted optic fibers. Stimulation remained on while the animal remained in the zone and stopped as soon as the animal left the inhibition zone (Fig. 2D).

Striatal CINs specificity. To resolve the specificity of the transgenic ChAT-Cre line, we analyzed striatal slices from a ChAT-Cre mouse crossed with an Ai35 mouse (ChAT-Cre:Ai35, Madisen et al., 2012) (Fig. 2A), and four ChAT-Cre mice infected with eYFP (used as control animals in behavioral experiments) (Fig. 2B). These animals were terminally anesthetized with ketamine/xylazine (85 and 15% respectively) and transcardially perfused with 0.1 M PBS followed by 4% paraformaldehyde (PFA). The brains were extracted and left in post-fix overnight. Afterward, 50 μm coronal slices were obtained (vibratome; 3000 Tedpella). For immunostaining, after washing with 0.1 M PBS, sections were incubated with primary antibody overnight at room temperature (anti-ChAT antibody, Millipore, No. AB144P, diluted 1:1000 in 0.2% Triton X-100: 0.1 M PBS). On the next day, slices were washed five times with 0.1 M PBS and incubated at room temperature with secondary antibody (Alexa Fluor 594, Thermo Fisher Scientific, A-11080, diluted 1:1000) for 2 h. Sections were washed five times with 0.1 M PBS and mounted. Images were acquired with a confocal microscope (Zeiss LSM 710, ZEISS) and quantification of neurons was carried out in the DMS (850 μm × 850 μm regions).

In vivo electrophysiology.

To investigate the effects of optogenetic manipulations on CINs activity, ChAT-Cre animals ($n = 2$) were injected unilaterally with 500nL of Arch3.0-eYFP (AAV5-EF1a-DIO-eArch3.0-EYFP) in the DMS and after 1 week implanted with a custom made optrode. For the optrode, we affixed an optic fiber with acrylic cement to a movable microwire bundle (16 Channels) manufactured by Innovative Neurophysiology, Durham, NC, in a way that both tips pointed together; Fig. 4A. After allowing 3 weeks for opsin expression, data acquisition was conducted in a square acrylic chamber (open field) of 40 cm × 40 cm (walls 30 cm high). Electrophysiological activity was acquired at 30 kHz (CerePlex Direct, Blackrock Microsystems, UT) and processed with a high-pass filter (750 Hz) to extract spike activity (see Fig. 4B, C). Three patterns of 556 nm light (continuous, 3 ms–10 Hz, and 20 ms–10 Hz pulses) were applied for 4 seconds (s), for at least 30 trials. We used four seconds since this duration covered 96% of the entire distribution of time that the animals were in the inhibition zone (0.9 ± 0.1 s, $n = 7$ animals). Units were sorted online through manual thresholding and manual hoop algorithms (Blackrock Microsystems, UT) and offline with a principal component analysis algorithm (Offline Sorter, Plexon). Data was exported to MATLAB and further analyzed through custom scripts. Spike activity of each unit was aligned to the onset of light delivery. We applied column-wise Receiver Operating Characteristic (ROC) curve analysis with a 200 ms sliding window moving in 100 ms steps, unless otherwise stated (baseline was from −1 to −0.8 s from light onset). We chose a 200 ms window since the average firing rate of medium spiny neurons is about 5 Hz (around 1 spike every 200 ms). The 100 ms sliding window was chosen since shorter windows did not improve the detection of the overall modulations. Units with ROC values < 0.2 during 1 s of continuous light inhibition were photo-identified as cholinergic ($n = 2$, at least 20 trials; see Fig. 4D). Latency to inhibition was considered as ROC < 0.3 with a 200 ms sliding window in 1 ms steps. Mean firing rate was computed by averaging the number of spikes during the 4 s of light delivery and the 4 s preceding it per trial; the Kruskal–Wallis method was used to test equality.

To analyze the periodicity of spike activity in the frequency domain, discrete firing rate was obtained by binning time and counting spikes with dt = 10 ms from −4 s (basal) to 4 s from light onset (light inhibition). Fast Fourier Transform was performed per trial and averaged across trials (Fig. 4F). Inter-spike intervals were computed for the 4 s preceding and the 4 s of light inhibition to create the histograms in Fig. 4H. These histograms were expressed as cumulative density functions (CDF) and tested for equality with a Kolmogorov–Smirnov test. This same procedure was

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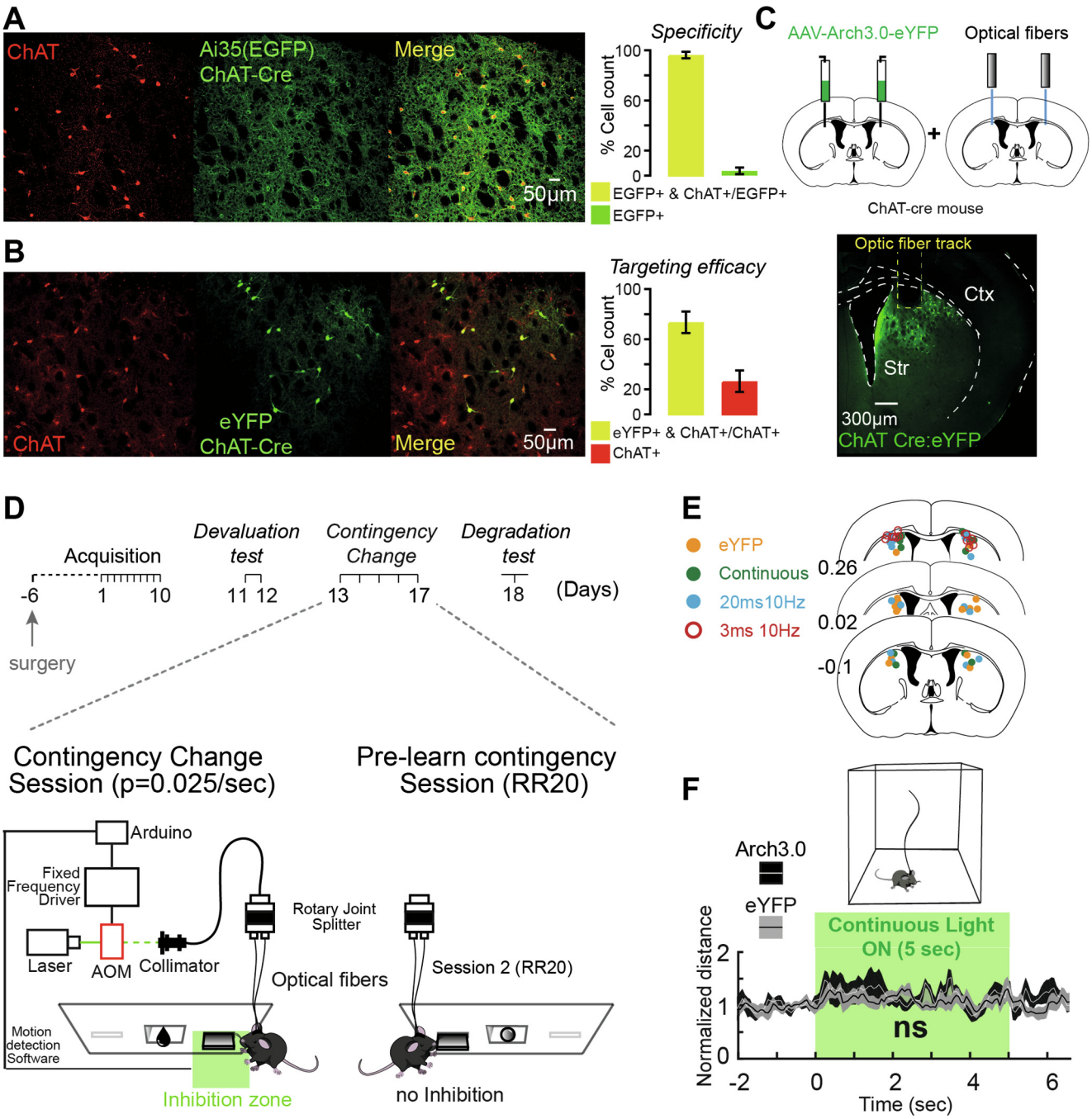

Fig. 2. Strategy for the optogenetic manipulation of striatal cholinergic interneuron during a contingency change. (**A**) From left to right, Cholinergic neurons tagged with Alexa 594 (ChAT, Red-left), EGFP expression by Ai35 mouse (Ai35 (EGFP), Green-middle) and merge (Merge, Yellow-right). The bar represents the specificity of the transgenic line: percentage of EGFP+ or eYFP+ (from the ChAT-Cre:Ai35 crossbreed and virally transfected a ChAT-Cre: eYFP mice) that were co-labeled as ChAT+. (**B**) Photos similar to A, this time by viral transfection (AVV) of eYFP in the ChAT Cre mice. Bar represents targeting efficacy evaluated as [eYFP+ & ChAT+]/ChAT+. (**C**) Schematic representation of viral transfection of Archaerhodopsin (AAV-Arch3.0) and optic fibers implantation in dorsomedial striatum (top), and representative slice of optical fiber track in a ChAT-Cre: eYFP mouse (bottom). (**D**) Timeline of optogenetic manipulation during the contingency degradation sessions (green square indicates inhibition zone). (**E**) Location of optical fiber tracks implanted in experimental groups. (**F**) Open field tracking of general movement: horizontal displacement from ChAT-Cre animals expressing either Arch3.0-eYFP or only eYFP. No difference was detected between these groups. $p > 0.05$ Mann Whitney *U* test.

applied to all non-PhotoID units recorded. For non-PhotoID units, firing rate was expressed as Z-score (mean and sigma was calculated from the basal period). A Wilcoxon (paired) test was performed (basal vs light inhibition periods) and those with $p < 0.01$ were classified as modulated.

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Statistical analyses

All statistical analyses were performed using Graph Pad 7 and MATLAB 2016. A criterion of $p < 0.05$ was used to significance, unless otherwise stated. Data are presented as mean ± standard error, unless otherwise specified in the text.

RESULTS

To identify whether the activity of the striatal CINs is required to encode a change in the contingency between an action and its outcome, we first implemented a protocol in which mice were trained to acquire specific contingencies between two actions and two outcomes (A1 → O1 and A2 → O2) (Balleine and Dickinson, 1998; Bradfield et al., 2013; Matamales et al., 2016). We later manipulated the activity of the striatal CINs while one of the action → outcome contingencies was degraded.

Mice identify changes in action outcome-contingencies

To be able to change a contingency between an action and its outcome, we first needed to train animals to perform goal directed actions. Training mice to press a lever with a random ratio schedule induces animals to press in a goal-directed manner (Balleine and Dickinson, 1998; Corbit and Balleine, 2000; Hammond, 1980; Yin et al., 2005). Accordingly, we trained mice in a two actions–two outcomes protocol that induces animals to perform goal-directed levers pressing (Bradfield et al., 2013; Matamales et al., 2016; Yin et al., 2005). The training protocol consisted of exposing animals to one of two levers in an operant box for two sessions each day. During session 1, only the lever to the left (or to the right, animals were counterbalanced) of a food magazine was presented. During session 2 only the second lever on the opposite side of the food magazine was presented. Throughout the first three days of training, a single lever press delivered a reinforcer [a pellet in Session 1 (action one → outcome one; A1 → O1) or a drop of sucrose in session 2 (action 2 → outcome two; A2 → O2)]. Afterward, the contingency to obtain a reinforcer was modified to a random ratio (RR) schedule for ten days; two days in RR5, two days in RR10 and six days in RR20 (pressing ∼5, ∼10 or ∼20 times delivered a reinforcer, respectively) (Fig. 1A). During the acquisition of this RR contingency, animals increased the number of presses (mean presses in session 1 for the two outcomes = 114 ± 9, *versus* in session 10 = 378 ± 34, $n = 9$ animals, $p < 0.0001$, Wilcoxon test; Fig. 1A bottom panel), as well as the press rate for the two outcomes (presses/min session 1 = 5.9 ± 0.6 *versus* session 10 = 25.3 ± 5.2, $n = 9$ animals, $p < 0.0001$, Wilcoxon test). Afterward, to evaluate whether the animals were pressing the levers based on the outcome of the action on each lever, each animal was subjected to a pair of devaluation tests. During each day of the devaluation test, animals were satiated with one of the two outcomes previously received (Fig. 1B) and immediately placed in the operant chamber for 10 minutes. During this time, the animals were presented with the two levers and the presses in each of the levers were counted. The devaluation test is done in extinction, so there is no delivery of a reinforcer. If the animals had correctly distinguished the outcome of pressing each of the levers, then they would perform more lever presses on the lever associated with the outcome that was not devalued. Accordingly, the devaluation test showed that animals pressed more times the lever that delivered the outcome that was not devalued [mean presses in the devalued outcome = 39 ± 10 *versus* the non-devalued outcome = 102 ± 16, $n = 9$ animals, $p < 0.005$, Wilcoxon test (paired comparison) and Mann Whitney *U* test (bars); Fig. 1B bottom panel].

The update in the performance of rodents according to a change in contingencies has been previously documented with action-outcome specificity (Bradfield et al., 2013; Matamales et al., 2016; Yin et al., 2005). Following these previous reports, and in order to internally control the update of instrumental contingencies, each animal from the group described in Fig. 1A, B was exposed to contingency degradation training. During the degradation training each animal was trained for two sessions a day. One session kept the RR20 contingency [A2 → O2 (contingency RR20)], and in the other session the contingency was degraded [(A1 → O1 (contingency degraded)]. In the degradation session the probability to receive a reinforcer was 0.025 every second that the animal did not press the lever (all experiments were counterbalanced, see Methods). Fig. 1C shows that the press rate during the session where the contingency was changed decreased significantly as compared to the press rate during the sessions with no change in contingency (press rate during the last day of RR20 = 18.4 ± 5.5 *versus* the press rate in the last day of contingency degradation = 3 ± 1.3, $n = 9$ animals, $p < 0.05$, Wilcoxon test; Fig. 1C bottom panel). The next day, a test in extinction was performed to evaluate whether the animals adjusted their behavior to the change in contingency (Fig. 1D). This test consisted of exposing the animals to the two levers without delivery of a reinforcer. Fig. 1D (bottom panel) shows that mice performed fewer presses on the lever with the changed (degraded) contingency as compared to the presses on the lever that did not change contingency (non-degraded) [mean presses, degraded = 30 ± 6 *versus* non-degraded = 151 ± 31, $n = 9$ animals, $p < 0.005$, Wilcoxon test (paired comparison) and Mann Whitney *U* test (bars)].

The data presented up to here replicates the previously reported capabilities of mice to identify an action-outcome contingency change (Bradfield et al., 2013).

Targeting of the cholinergic striatal interneurons to express proteins of interest

In order to ask whether the striatal CINs contribute to the encoding of a change in instrumental contingency we used ChAT-Cre mice. ChAT-Cre mice express the Cre recombinase under the expression of the choline acetyltransferase promoter, a molecular marker of cholinergic neurons (Rossi et al., 2011). To evaluate the

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specificity of the ChAT-Cre mouse line to target cholinergic striatal neurons, we crossed these mice with a reporter mouse line that expresses EGFP in cells containing Cre (Ai35 mouse line) (Madisen et al., 2012), or transfected ChAT-Cre mice with a virus to express eYFP in a Cre-dependent manner. Slices were immune-stained for positive ChAT expression. The quantification of EGFP+ or eYFP & ChAT+ cell bodies in randomly selected Z-stacks of the DMS from one ChAT-Cre:Ai35 mouse and four ChAT-Cre:eYFP mice (covering coordinates: AP: −0.1 to 0.15, one 850-μm quadrant per slices) showed that the specificity to target cholinergic striatal interneurons (defined as a EGFP+ & ChAT+/EGFP+ cells) was 96 ± 2% (n = 1 mouse ChAT-Cre:Ai35 and 4 mice ChAT-Cre:eYFP, 5 slices from the ChAT-Cre:Ai35, 7 slices from the ChAT-Cre:eYFP, Fig. 2A). This verification replicated previous reports (English et al., 2011; Maurice et al., 2015; Zucca et al., 2018), and allowed us to use the ChAT-Cre mice to virally express proteins of interest in striatal CINs in the DMS. To evaluate the transfection rate in the center of the viral infections, we quantified the eYFP + & ChAT+/ChAT+ cells, observing an average of 73 ± 8% of cells labeled (n = 4 mice; seven slices, Fig. 2B).

Optogenetic manipulations of striatal CINs impaired the identification of an instrumental contingency change

Once we evaluated the specificity of the transgenic mouse line, a group of ChAT-Cre mice was bilaterally injected with an adeno associated virus (AAV) to express Archeorhodopsin (Arch3.0), an opsin that when illuminated with green light hyperpolarizes neurons (Chow et al., 2010; Mattis et al., 2011), and implanted optic fibers 100 μm above the injections as described in the methods (Fig. 2C). Allowing six days for recovery, we trained these animals as previously described in Fig. 1. The hypothesis to test in this group of animals was that the activity of the cholinergic neurons was required for the proper identification of a change in an action-outcome contingency. We therefore adapted a video tracking system to the operant box and set up the condition that during the sessions of the contingency degradation, every time that animals were in the zone of the action (in front of the lever: inhibition zone) they received green light illumination into the DMS through the implanted optic fibers to manipulate CINs (Fig. 2D, see Methods). The positions of the fibers' tips from all groups of animals were verified to be in the DMS (Fig. 2E). Like wild-type mice (Fig. 1) implanted ChAT-Cre animals expressing Arch3.0-eYFP also increased their lever pressing, throughout the acquisition of the action → outcome contingencies (Fig. 3A, left panel; mean presses in session 1 for the two outcomes = 72 ± 10 *versus*, in session 10 = 344 ± 35, n = 6 animals, $p < 0.001$, Wilcoxon test). They were also sensitive to the devaluation test [Fig. 3A, second column from left to right, *devaluation test*; mean presses in the devalued outcome = 19 ± 5 *versus* the non-devalued outcome = 116 ± 26, n = 6 animals, $p < 0.005$, Wilcoxon test (paired comparison) and Mann Whitney U test (bars)]. Consistent with our hypothesis, inhibition of ChAT-Cre:Arch3.0-eYFP cells in the DMS was sufficient to impair mice from adjusting to the change in contingency as reported by the degradation test (mean presses on the lever of contingency degradation $_{\text{Arch3.0 continuous}}$ = 96 ± 39 *versus* presses on the lever associated with RR20 (non-degraded) $_{\text{Arch3.0 continuous}}$ = 137 ± 32, n = 5 animals, $p > 0.2$, Wilcoxon test (paired) and Mann Whitney U test (bars), Fig. 3A, right column, *degradation test*).

Nevertheless, we were concerned about confounding factors such as the possibility that the striatal cholinergic cells could present rebound firing activity finishing the optogenetic inhibition (Atallah et al., 2014; Zucca et al., 2018, English et al., 2011), or that the duration of illumination (mean time per bout of presses = 0.9 ± 0.1 s) could generate a lesion (Gao et al., 2018; Stujenske et al., 2015). Therefore, two different cohorts of ChAT-Cre:Arch3.0-eYFP animals were evaluated, this time receiving brief pulses of light at 10 Hz; one group received 10 Hz stimulation with a pulse size of 20 ms, (Fig. 3B) and the second group 10 Hz with 3 ms of pulse size (Fig. 3C). 10 Hz was chosen as we thought it is within a range to avoid the putative 200 ms pause in activity of CINs, which has been suggested to be key for the contribution of CINs to the update in contingencies (Kim et al., 2019; Goldberg and Reynolds, 2011; Aosaki et al., 1994); 3 and 20 ms were chosen as the former was on the lower and the latter on the highest probability to cause inhibition according to the inactivation parameters of Arch (Mattis et al., 2011). Remarkably, only the group that received 20 ms–10 Hz pulses of light showed an impairment in the identification of the change in contingency (mean presses, contingency degraded $_{\text{Arch3.0 20ms–10Hz}}$ = 171 ± 44 *versus* contingency non-degraded $_{\text{Arch3.0 20ms–10Hz}}$ = 190 ± 63, n = 8 animals, $p > 0.5$, Wilcoxon test (paired) and Mann Whitney U test (bars), Fig. 3B, right panel, *degradation test*), an effect that was not observed either in Ach3.0-eYFP animals receiving 3 ms–10 Hz pulses (mean presses, contingency degraded $_{\text{Arch3.0 3ms–10Hz}}$ = 65 ± 15 *versus* contingency non-degraded $_{\text{Arch3.0 3ms–10Hz}}$ = 238 ± 48, n = 7 animals, $p < 0.05$, Wilcoxon test (paired) and Mann Whitney U test (bars), Fig. 3C, right panel, *degradation test*), or in control ChAT-Cre animals expressing only eYFP subjected to 20 ms–10 Hz pulses of light (mean presses, contingency degraded $_{\text{eYFP 20ms–10Hz}}$ = 60 ± 21 *versus* contingency non-degraded $_{\text{eYFP 20ms–10Hz}}$ = 275 ± 76, n = 8 animals, $p < 0.05$, Wilcoxon test (paired) and Mann Whitney U test (bars), Fig. 3D, right panel, *degradation test*). No difference in the identification of the contingency change between the animals expressing Arch-eYFP subjected to the 3 ms–10 Hz protocol and the ones expressing eYFP was detected (mean presses in degradation test, contingency degraded $_{\text{Arch 3ms-10Hz}}$ = 65 ± 15 *versus* contingency degraded $_{\text{eYFP 20ms-10Hz}}$ = 60 ± 21 and contingency non-degraded $_{\text{Arch 3ms-10Hz}}$ = 238 ± 48 *versus* contingency non-degraded $_{\text{eYFP 20ms-10Hz}}$ = 275 ± 76; n = 7 and 8 animals for each group respectively, $p > 0.5$, Mann Whitney U test).

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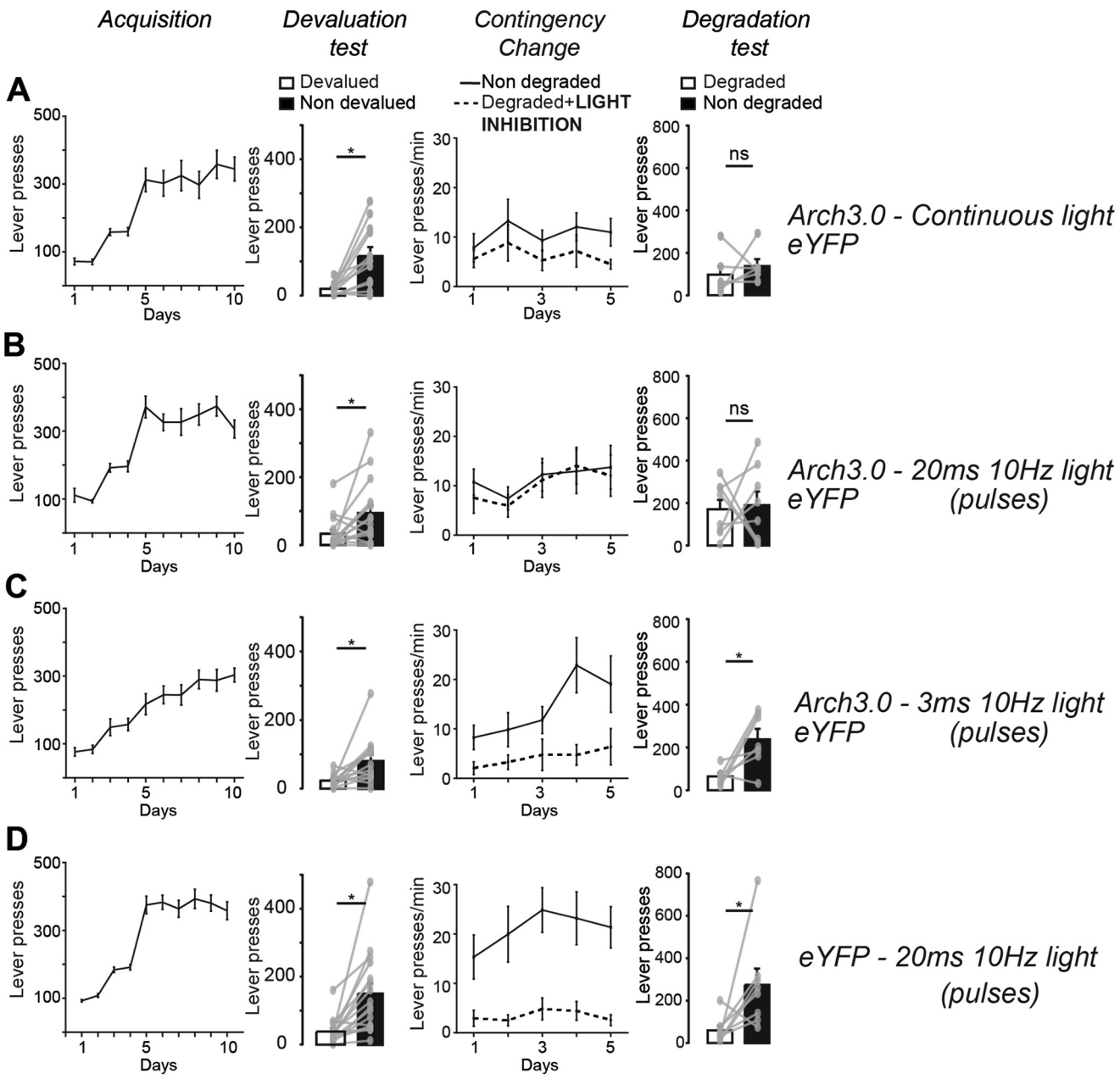
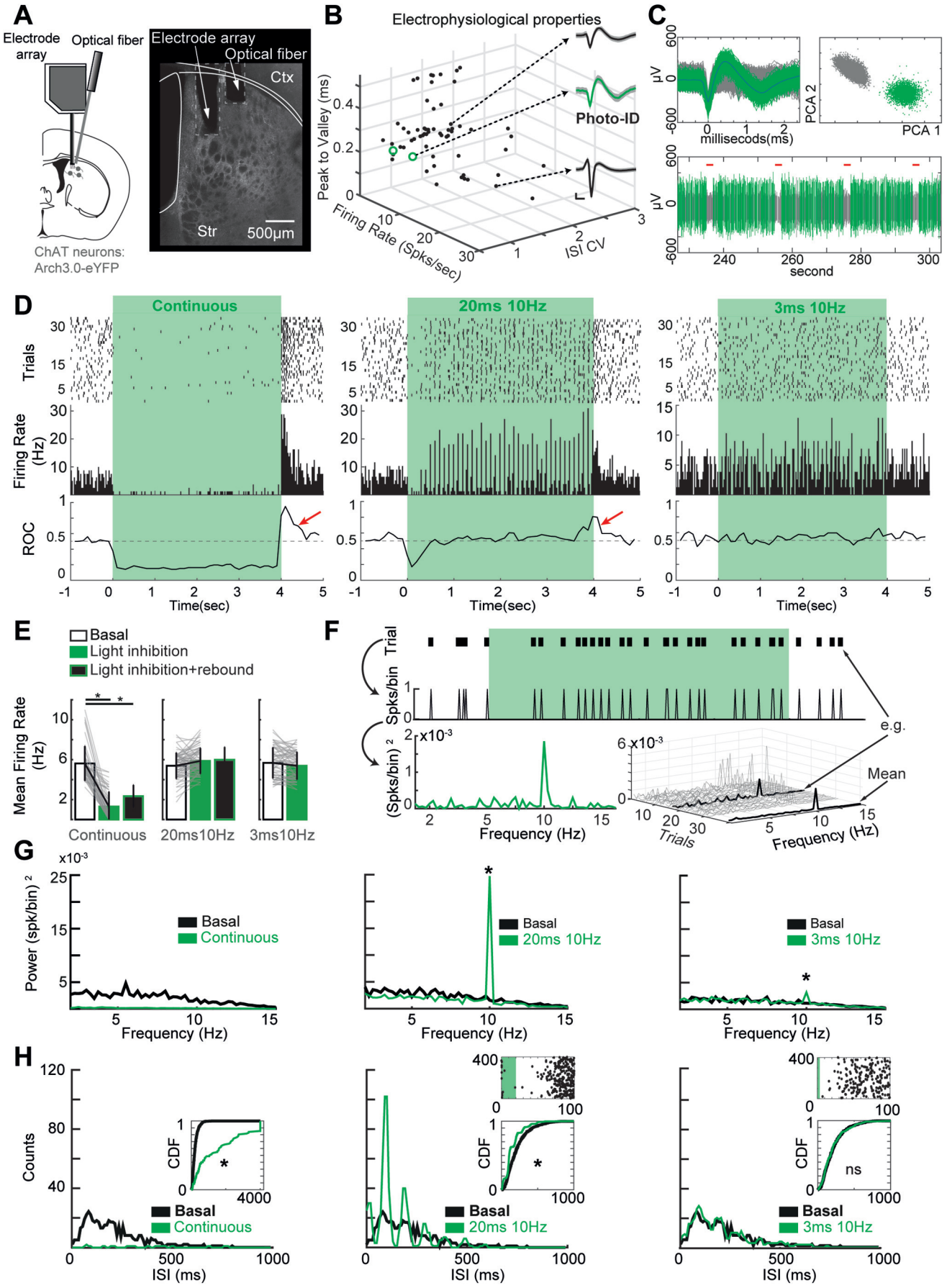


Fig. 3. Optogenetic perturbation of striatal cholinergic interneurons impairs the detection of a contingency change. (**A**–**C**) From left to right, similar training as described in Fig. 1 for three cohorts of ChAT-Cre: Arch3.0-eYFP animals in which different protocols of light inhibition were tested during the session of contingency change (degraded). Continuous light (**A**) and pulsed protocols (**B**, **C**). Note that continuous and pulsed 20 ms at 10 Hz impaired the identification of the contingency change as reported by the equal number of presses on each lever during the degradation test. (**D**) Similar to **B** in a control group of ChAT-Cre animals expressing only eYFP. Not significant (ns) in **A**, **B**, $p > 0.05$ for both, Wilcoxon (paired) and Mann Whitney *U* tests (bars). **A**–**D** *$p < 0.05$, for Wilcoxon (paired) and Mann Whitney *U* tests (bars).

Green light illumination inhibits/perturbs activity of striatal CINs expressing Arch3.0

Given that different patterns of green light on the DMS–CINs impaired the identification of the instrumental contingency change, we sought to evaluate the effects of these manipulations on the activity of these neurons in vivo. In order to do so, two ChAT-Cre mice were virally transfected to express Arch3.0 and implanted with a movable optrode (Atallah et al., 2014; Tecuapetla et al., 2014; see Methods) (Fig. 4A). After recording 10 sessions (5 different positions per animal), two striatal units (1 in each animal) were identified as ChAT-Cre-Arch3.0+ (see Methods) and were recorded under the different protocols of green light illumination. Fig. 4B, C show the electrophysiological properties of all recorded units and the raw data from one of the photo identified CINs, respectively. Interestingly the parameter that seems to distinguish the CINs from the rest of striatal neurons is their coefficient of variation (Fig. 4B; Atallah et al., 2014: Stalnaker et al., 2016; Zucca et al., 2018). Fig. 4D shows the raster plots and peri-stimulus histograms of the


A
Electrode array
Optical fiber
Electrode array
Optical fiber
Ctx
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ChAT neurons:
Arch3.0-eYFP
B
Electrophysiological properties
Peak to Valley (ms)
Firing Rate (Spks/sec)
ISI CV
Photo-ID
C
µV
millisecods(ms)
PCA 2
PCA 1
second
D
Continuous
20ms 10Hz
3ms 10Hz
Trials
Firing Rate (Hz)
ROC
Time(sec)
E
Basal
Light inhibition
Light inhibition+rebound
Mean Firing Rate (Hz)
Continuous
20ms10Hz
3ms10Hz
F
Trial
Spks/bin
(Spks/bin) ²
Frequency (Hz)
e.g.
Mean
Trials
G
Power (spk/bin) ²
Basal
Continuous
20ms 10Hz
3ms 10Hz
H
Counts
CDF
ISI (ms)
ns

spike activity from one of these units that was exposed to the three light protocols. During continuous light, the cells presented a sustained inhibition (latency to inhibition < 8 ms; ROC < 0.3 for the length duration of the continuous light) (Fig. 4D left panels). However, with the 20 ms–10 Hz protocol, the cells were inhibited only at the beginning of the stimulation (ROC < 0.3 during 370 ms; Fig. 4D: middle panels) and the 3 ms–10 Hz protocol showed no change in the ROC curve (Fig. 4D: right panel). Importantly, when comparing the mean firing rate during each light manipulation trial for the two units identified as ChAT-Cre-Arch3.0+, we observed an inhibition only for the continuous light exposure (mean rate was reduced from 5.6 ± 1.7 Hz to 1.3 ± 1.5 Hz, $p < 0.001$, Wilcoxon test, Fig. 4E: left panel; mean rate $basal_{20ms\ 10Hz}$ = 5.4 ± 1.3 Hz vs. $light_{20ms\text{-}10Hz}$ = 5.8 ± 1.2 Hz; mean rate $basal_{3ms\text{-}10Hz}$ = 5.7 ± 1.5 Hz vs. $light_{3ms\text{-}10Hz}$ = 5.4 ± 1.4 Hz, $p > 0.05$, Wilcoxon test, Fig. 4E: middle and right panels respectively). However, a closer look into the peri-event histogram, the 20 ms–10 Hz protocol seems to establish a firing pattern. Hence, we examined the periodicity of the firing rate through a Fourier decomposition of the spike activity during light exposure (Fig. 4G). This analysis revealed that both the 3 ms and the 20 ms 10 Hz protocols significantly favored a periodic firing pattern, with the 20 ms–10 Hz protocol revealing a stronger change in power at 10 Hz (comparison of the data per trial versus the baseline with no light, and comparison of trials between pulsed protocols, $p < 0.001$; Wilcoxon test, Fig. 4G). Furthermore, analyses of inter-spike-interval (ISI) histograms from the baseline *versus* the activity during the light exposure found differences in the continuous and the 20 ms–10 Hz protocols with respect to basal activity ($p < 0.001$, Kolmogorov-Smirnov test; Fig. 4H, insets). Thus, we identified that continuous and 20 ms–10 Hz illuminations of striatal cholinergic cells *in vivo* significantly perturb the firing pattern as compared to basal activity. Importantly the strongest manipulation (continuous illumination) did not affect general movement (comparison of 15 s, 5 s before, during and after light, moving window of one-second ChAT-Cre: Arch-eYFP vs. ChaT-Cre:eYFP, $n = 7$ animals, $p > 0.05$, Mann Whitney *U* test, Fig. 2F).

In conclusion, the data presented from the optogenetic manipulations and the electrophysiological recordings *in vivo* support the idea that besides the presence of the CINs, an alteration in their pattern of activity, during the encoding of the instrumental contingency, impairs the proper identification of the change.

DISCUSSION

Although CINs are a crucial component of the DMS and their presence and activity have been implicated in encoding changes of previously learned contingencies, to date no study has attempted to optogenetically manipulate their activity during a contingency change and evaluate its effects. In this study, we endeavored to undertake this challenge and documented that manipulations that inhibit or perturb the activity of DMS-CINs impair the identification of a contingency change.

Counterbalancing of outcomes

In order to evaluate a contingency change, we based our study on previously developed protocols (Balleine and Dickinson, 1998; Bradfield et al., 2013; Corbit and Balleine, 2000; Matamales et al., 2016; Yin et al., 2005). However, some differences should be noted. The establishment of the two action-two outcomes training protocol in this study has been shown to work in rats using pellets and sucrose as reinforcers (Bradfield et al., 2013; Yin et al., 2005). Nonetheless, during our first attempts to set up the training protocol, when using pellets *versus* 10% sucrose, once animals were trained in RR schedule their performance for sucrose decreased over days (data not shown). It was only when we used pellets *versus* 20% sucrose that animals engaged in the two sessions each day of training. We assume that increasing the caloric content in sucrose made the animals work for the two outcomes (Reilly, 1999). Importantly, all treatments were counterbalanced: for half of the animals in each group

Fig. 4. Different optogenetic illumination protocols inhibit or perturb striatal cholinergic interneuron activity in vivo in an open field. (**A**) Schematic of optrode; electrode array with optical fiber (left panel): Photomicrograph, coronal striatal section of a ChAT-Cre animal transfected to express Arch3.0-eYFP and implanted with an optrode (right panel). (**B**) Conventional 3D plotting of electrophysiological properties depicting peak to valley latencies, firing rate, and the coefficient of variation of inter-spike intervals (ISI-CV) as a method to classify striatal waveforms. Note that both Photo Identified (Photo-ID) ChAT-Cre Arch+ units (the two green circles come from each of the Photo-ID cells, one per animal recorded) lie in a similar place in this space. Scale of waveform insets: 100 microV, 100 micros. (**C**) Raw data of a Photo Identified unit. Upper Left shows the raw waveforms in a channel containing a Photo Identified unit. Upper Right, the first two principal components of the waveforms are enough to separate the Photo Identified unit from the noise cluster ($p < 0.001$, multivariate ANOVA). Lower: Waveforms are presented during a segment of time, showing that light illumination (red lines) inhibit firing of the unit. (**D**) Upper panels: raster plot of spike activity from a photo-identified striatal cholinergic interneuron aligned to light onset across different trials for the three light patterns tested: continuous, 20 ms at 10 Hz, and 3 ms at 10 Hz, respectively. Middle panels: peri-event histograms of the raster plots (bin = 25 ms). Bottom panels: ROC analysis for each light inhibition pattern. Red arrows show end of ROC significance for rebound bursting. (**E**) Mean firing rate during the 4 s of light stimulation and the 4 s preceding it (basal), per trial per unit recorded ($n = 2$ neurons) for each of the light protocols tested (*$p < 0.001$, Wilcoxon (paired) test). An additional bar is presented for units with rebound activity; mean firing rate from start of light inhibition until red arrow in panel **D** Lower (light inhibition + rebound) is reported. (**F**) Fourier analysis of spike trains pipeline. The spikes of one trial are transformed to a continuous signal through binning (dt = 10 ms). Fourier Transform is performed on the segment during light stimulation. This is performed for each trial and averaged. Means are presented in **G**. (**G**) Average frequency domain analysis of the spike trains during each light protocol and basal activity. (**E**) Inter-spike Interval histogram (ISI) for spike activity during each light stimulation protocol and basal activity (inset: cumulative density function CDF). Upper insets in Middle and Left are rasters showing spike activity aligned to the start of each illumination pulse (20 ms or 3 ms).

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the Outcome 1 was pellets, and for the other half, it was 20% sucrose. This becomes relevant when the contingency change was carried out, degrading the instrumental contingency in half of the animals for sucrose and the other for pellets. This design grants an internal control and strengthens its validity since, despite being different outcomes (sucrose or pellets), when degraded the animals decreased pressing for each outcome (Fig. 1C, D and Fig. 3A–D degradation training and degradation test).

The perturbation of the activity of striatal CINs impairs the encoding of the update in the action-outcome contingency

The specificity of the ChAT-Cre mice used in this study has been previously documented (Matamales et al., 2016; Maurice et al., 2015; Zucca et al., 2018) and confirmed to be above 95% for the specificity of the Cre line and above 85% for the viral expression of Arch3.0 in the DMS CINs (Fig. 2).

Delivery of continuous green light to DMS CINs inhibited their spiking activity during the degradation training, and impaired the identification of the change in contingency as reported by lack of distinction in the presses during the degradation test (Fig. 3A, right column). However, despite the *in vivo* recordings, we were concerned that the amount of continuous light that animals received during the contingency degradation training might be harmful to the cells across days. In order to alleviate this concern and based on a strategy to briefly activate Arch3.0 and decrease the amount of light exposure, just enough to alter to the spiking of CINs (Znamenskiy and Zador, 2013), we tested two pulsed protocols at 10 Hz variating the size of the light pulse (3 or 20 ms light pulses), during the change in contingency. 10 Hz was chosen as we thought it would impair the pause of 200 ms of CINs which has been suggested to be key of CINs during contingency updates (Kim et al., 2019; Goldberg and Reynolds, 2011; Aosaki et al., 1994). Note that we delivered the light patterned protocols through a laser beam controlled using an AOM, which allows microsecond modulations of the pattern of light (see Methods). From these two protocols of pulsed light, only the 20 ms pulses at 10 Hz but not the 3 ms pulses at 10 Hz impaired the identification of the change in contingency (Fig. 3B *vs.* 3C). Remarkably, when we recorded the activity of photo-identified CINs in vivo, both protocols favored a periodicity in their firing pattern at 10 Hz (Fig. 4G). Yet, the magnitude of power change induced by the 20 ms 10 Hz protocol as compared to the baseline-spiking pattern was higher (Fig. 4G). This increased periodicity of firing pattern significantly altered the overall firing pattern only in the 20 ms 10 Hz protocol, as evidenced by its effect in the inter-spike interval (ISI) histograms (Fig. 4H). A lack of a significant effect of the 3 ms 10 Hz inhibition in the ISI seems to be a consequence of weaker displacement in the ISI following the light inhibition (inserts above the CDF plots in Fig. 4H). Importantly, the additional control of testing a group of CINs-eYFP under the 20 ms–10 Hz protocol did not attenuate the identification of the contingency (Fig. 3D). It must be noted that the recordings presented here were performed outside of the behavioral task and attempt to provide a guide for what could happen in the manipulations during the behavioral paradigm.

Why does the time of inhibition was chosen around the performance of the action?

During the training of animals in a random ratio schedule (as we did here), which induces goal directed actions, the striatal activity around the performance of the action has its highest modulation around this moment (Gremel and Costa, 2013), perhaps because the action leads the consequence. Therefore we hypothesized that the modulation of CINs would be more important around the lever press to update actions in response to changes in contin-

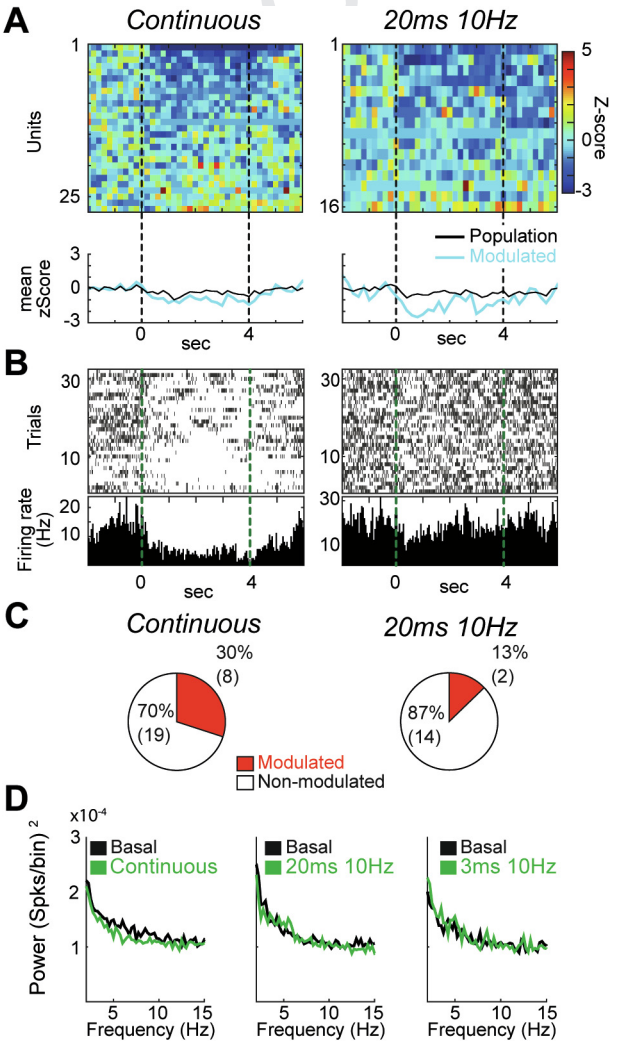

Fig. 5. Modulation of surrounding units due to stimulation of ChAT-Cre Arch+ striatal cells. (**A**) Top, heatmap showing Z-score of average firing rate before, during and after light inhibition as specified by dashed lines. Left, continuous light inhibition. Right, 20 ms at 10 Hz light inhibition. Bottom, average Z-score of all units (population), and average Z-score of modulated units 4 s during light inhibition vs 4 s before (basal) ($p < 0.01$, Wilcoxon (paired) test). (**B**) Example of modulated units for each light inhibition protocol. (**C**) Proportion of units modulated for each light inhibition protocol. (**D**) Average frequency domain analysis (as explained in Fig. 4**F**) of the spike activity of all units during each light protocol.

gencies. An additional thought to present the inhibition around the performance of the action is based on the fact that the activity modulation of CINs, in classical conditioning late in training, has been documented in response to a stimulus that predicts a reward (Ravel et al., 2003; Apicella et al., 1997). We hypothesized that in instrumental conditioning, as we used here, the CINs modulation may happen during the action that precedes the consequence. Finally given the mechanisms through which the Acetylcholine of CINs may act (muscarinic receptors, slower than ionotropic mechanism) we also hypothesized that the modulation of CINs could be more relevant time before the performance of the action.

Nonetheless and despite that our recording of the CINs and surrounding striatal cells during the optogenetic inhibitions returned very quickly to baseline we could not rule out that our inhibitions of CINs may have had an impact at the moment of the consequence, therefore further investigations should explore if the inhibition of CINs during the consequence of the action may also impair the update of action to changes in instrumental contingencies.

Was the pulsed inhibition of CINs what impaired the update of action to the change in contingency or the transient inhibition at the beginning (<200 ms) or the rebound at the end of the inhibition?

Since it has been documented that the minimum time of light inhibition of CINs to modulate the spiking of striatal neurons is around 400–500 ms (Zucca et al., 2018, English et al., 2011; Witten et al., 2010), and in our analyses of the modulation of the surrounding striatal cells we also observed modulation of neurons as fast as in 200 ms (Fig. 5) we could not rule out the possibility that it is the small transient inhibition at the beginning of the 20 ms–10 Hz pulsed protocol what impairs the update of the action to the change in contingency, nevertheless it must be noted that animals toke ≈0.6 s from the moment that the inhibition was switched on to the moment they pressed the lever, meaning that by the time the animals were performing the action the CINs were already under pulsed inhibition.

Regarding the rebound, as expected a minor rebound in the pulsed protocol was observed as compared to the rebound in the continuous inhibition (red arrows panel D, Fig. 4), interestingly no effects was observed on the surrounding striatal cells during the moment of rebound in the pulsed protocol. Nevertheless an effect of the rebound on variables that are not reported by the spiking of surrounding cells could not be discharged (Mohebi et al., 2019). Therefore, and considering that our recordings were performed out of the behavioral task, our recordings suggest that the pulsed protocol would not allow the proper spiking of CINs, which in turn would not allow its proper modulation in the spiking of surrounding striatal cells.

In conclusion, the data from the optogenetic manipulations and the electrophysiological recordings from the photo-identified CINs support a model in which the presence of the striatal cholinergic cells in the DMS, and their activity during a change in an instrumental contingency, is essential for the appropriate identification of a contingency change.

How can the DMS–CINs contribute to the appropriate identification of an instrumental contingency change?

The activity of CINs in the DMS has been suggested to be preferentially modulated by their inputs from the thalamus (Brandfield, 2003; Ding et al., 2010). The mechanism by which the striatal CINs may act on the update of behavior in response to an instrumental contingency change has been suggested to be preferentially through the control of the activity of the striatal projection cells and/or the modulation of synaptic striatal inputs (Bonsi et al., 2008; Ding et al., 2010; Threlfell et al., 2012; Zucca et al., 2018).

One general idea is that either the activation (through direct modulation of muscarinic receptors or feedforward inhibition; Ding et al., 2010; English et al 2011) or the inhibition of CINs induces the inhibition of the striatal projection cells (Zucca et al 2018), although depolarizing actions of striatal Ach have been documented (Bonsi et al., 2008; English et al., 2014; Galarraga et al., 1999). For the mechanism of inhibition, studies in brain slices have suggested that an increase in the activity of CINs, mediated by a thalamic depolarization, results in a temporal window in which the striatal projection neurons from the indirect pathway (SPNi) are preferably depolarized by coordinated cortical inputs arriving at the same time (Ding et al., 2010). The depolarization of the SPNi in the DMS suppresses movements and evokes avoidance responses (Kravitz et al., 2010; Kravitz et al., 2012), the inhibition of SPNi is expected to favor movements, and be reinforcing, which if happening in our experiments may interfere with the identification of the change in contingency. To evaluate this possibility, we measured the general locomotion of animals while manipulating the activity of CINs in an open field arena (Fig. 2F). This measurement suggested that the manipulation of striatal CINs did not generate a change in general movements, consistent with previous reports (Maurice et al., 2015); also comparing the press rate during the first day of degradation did not show differences between the inhibited animals vs. their controls. In a second attempt to document the modulation of striatal cells by CINs activity, in our experiments, we estimated the firing rate of surrounding cells. A total of 63 striatal units were recorded, however, only 26 units crossed the criteria of being close to the photo ID CINs (either recorded on the same session or sessions 50 μm apart, Fig. 5). This analysis revealed that 30% and 13% were significantly (negatively) modulated during the continuous and the 20 ms at 10 Hz protocols, respectively (Fig. 5A–C).

Although it should be highlighted that this last data was not acquired during the behavioral training (it was acquired in an open field), it argues in favor of an online modulation of CINs on the striatal microcircuits, perhaps through the activation of metabotropic receptors (Zucca et al., 2018). However, the offline or long-term modulation of synaptic inputs cannot be disproved (Bonsi et al., 2008; Ding et al., 2010; Threlfell et al., 2012). Finally, the possibility that the increase in periodicity observed in the photo-

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identified CINs could emerge from light artifacts is ruled out since no similar modulation in periodicity of the surrounding striatal cells was observed (Fig. 5D).

In summary, as a mechanism of action, the perturbation in the spiking activity of CINs impairs the update of actions to a change in contingency, since we did not detect changes in the general locomotion of animals by the inhibition/perturbations of CINs. The observation of online modulation of spiking in the surrounding striatal units from our data, support a model in which the striatal CINs exert an online effect on striatal circuits, which allow the proper identification of an instrumental contingency change.

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DECLARATION OF INTEREST

None.

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