Chronic downregulation of parvalbumin-positive cell activity accelerates early perceptual learning but impairs end performance

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Abstract

Parvalbumin-positive (PV+) interneurons are major regulators of cortical experience-dependent plasticity. Using an adaptive auditory discrimination task, we found that perceptual learning is associated with a transient downregulation of PV expression in primary auditory cortex (A1), as previously shown in motor and hippocampal cortex. Chronic chemogenetic manipulation of A1 PV+ interneurons during training changed the rate of acquisition of new skills; such that up-regulation of PV+ cell activity accelerated perceptual learning, but reducing their activity resulted in slower learning. However, both interventions resulted in impaired perceptual acuity by the end of training, relative to controls. These findings suggest that, whereas reduced PV+ cell function may facilitate training-induced plasticity early in training, a subsequent increase in PV+ cell activity might be needed to prevent further plastic changes and consolidate learning.

Introduction

Adult brain plasticity is essentially restricted to the context of goal-directed behavior requiring sustained attention and leading to some form of reward or punishment (Blake et al., 2006; Polley et al., 2006; Sadahiro et al., 2016). Learning-induced cortical remodeling requires a change in the local excitatory/inhibitory (E/I) balance (Takesian and Hensch, 2013; Kuchibhotla et al., 2017), which is partially regulated by parvalbumin-positive (PV+) cells (Hu et al., 2014; Caroni, 2015), the most prevalent type of cortical inhibitory interneurons (Ouellet and de Villers-Sidani, 2014).

Recent studies have uncovered PV expression as a reliable proxy for cellular function, and have shown that cortical PV staining intensity is tightly correlated with the degree of experience-dependent plasticity (Donato et al., 2013; Karunakaran et al., 2016; Rowlands et al., 2018). Case in point, motor and spatial memory training are associated with region-specific—in primary motor cortex and hippocampus, respectively—shifts in PV 'network configuration', such that initial incremental trial-and-error learning is accompanied by reduced PV expression and a low E/I synaptic-density ratio; whereas expertise is associated with an increase in both PV expression and E/I synaptic-density ratio (Donato et al., 2013; Karunakaran et al., 2016). However, whether similar mechanisms regulate adult plasticity in primary sensory cortex remains unknown. And although these studies show that acute manipulation of PV+ interneuron activity can affect the rate of learning, the effects of sustained modulation of PV-inhibition on the acquisition and consolidation of new skills have not been documented.

To address these gaps in knowledge, we trained rats on an adaptive sound discrimination task and measured PV expression in primary auditory cortex (A1) at different time points during training. We then used chemogenetics to selectively and chronically manipulate PV+ cell activity in A1 during the same training task. Finally, we measured the functional outcomes of concurrent training and PV+ cell manipulation, and their relationship with longitudinal behavioral performance. Our findings shed new light on the inhibitory regulation of adult experience-dependent plasticity and have the potential to spur new lines of research aimed at improving learning in health and disease.

Results

Transient downregulation of PV expression associated with training

In sensory systems, improved perception can be achieved through practice in specific sensory discrimination tasks, a process known as perceptual learning (PL) (Caras and Sanes, 2017). To test whether PL is associated with changes in PV expression in A1 we trained rats using an adaptive auditory oddball discrimination task (**Figure 1A**). In this go/no-go behavioral paradigm, rats were rewarded for correctly identifying the presence of a deviant (oddball) tone in a short sequence of otherwise identical (standard) tones presented at 4 pulses per second (p.p.s.). The behavioral task followed a staircase procedure (one-up/one-down) with six levels of difficulty. Rats' performance improved steadily over 10 weeks with 5-6 one-hour sessions per week (**Figure 1B**). As expected for a go/no-go training, both hit rate and false-positive (FP) rate increased during early stages of training. On average, FP rate peaked at ~8 sessions, followed by a steady decline as rats learned to inhibit responses to non-target stimuli. We therefore chose this time point in training to assess the relationship between PV expression and learning during the early training phase.

As previously reported in other cortical areas (Donato et al., 2013; Karunakaran et al., 2016), we found downregulation of PV expression during early stages of training (**Figure 1C**-**D**, which likely facilitates modifications in cortical circuits that are needed for incremental trial-and-error perceptual learning (Happel et al., 2014; Khan et al., 2018). Conversely, expertise upon completion of training was associated with high PV expression, which might promote the consolidation of training-induced changes and prevent further circuit remodeling (Caroni, 2015; Karunakaran et al., 2016).

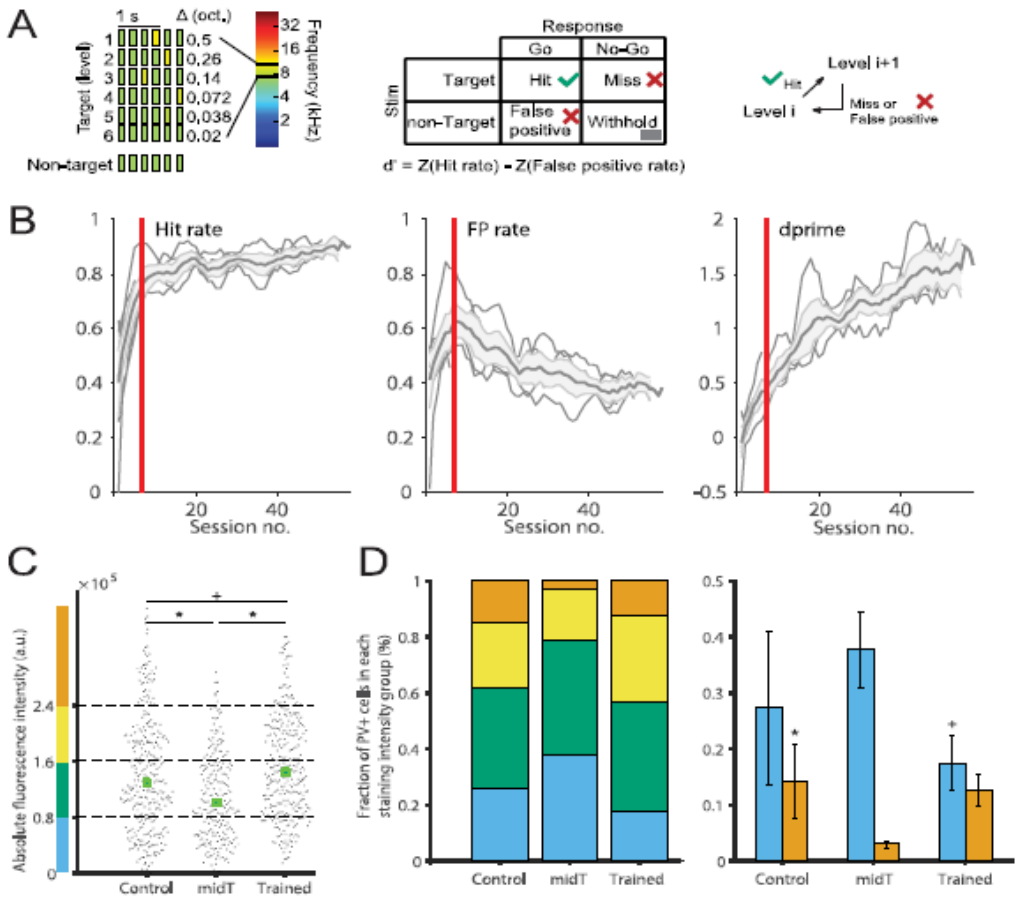

Figure 1. Effect of training on A1 PV expression. (**A**) *Left*, the difficulty level was increased progressively by reducing the frequency difference (Δfreq) between oddballs and standards after one hit. *Right*, lack or response to a target (miss) or a response to a series of 6 identical tones (false positive) resulted in a time-out and a decrease in the level of difficulty (staircase one-up, one-down). (**B**) Training performance. Mean hit rate, false-positive (FP) rate, and d' over a period of 60 sessions. Kruskal-Wallis test was used to assess the effect of condition (Naive (Control), mid-training (midT) and late training (Trained); χ^2 and p values are reported); values of follow-up multiple comparisons were corrected with Tukey's post-hoc test. (**C**) Overall PV+ staining intensity ($\chi2(2) = 45.01$, $p < 0.001$; Control vs midT, $p = 0$, Control vs Trained, $p = 0.057$, midT vs Trained, $p = 0$). (**D**) Cumulative (*left*) and relative (*right*) distribution of PV+ cell staining intensity for each condition. Further analysis for each intensity level revealed a significant effect of group on the proportion of high-intensity PV+ cells ($\chi2(2) = 6.54$, $p = 0.038$; Control vs midT, $p = 0.032$) and a near-significant effect of group on the proportion of low-intensity PV+ cells ($\chi2(2) = 4.67$, $p = 0.09$, Trained vs midT, $p = 0.08$). * $p < 0.05$, + $p \leq 0.09$.

Enhanced rate of procedural learning with sustained PV+ cell inactivation

Experimentally-induced changes to PV-network prior to training have opposite effects such that decreasing or increasing PV+ cell activity results in accelerated or delayed learning, respectively (Donato et al., 2013). However, the long-term effects of sustained PV+ cell manipulation

throughout training remain unknown. To tackle this question, we trained adult rats transfected with an inhibitory or excitatory DREADD [pAAV-hSYN-DIO-hM4D(Gi)-mCherry, "PV_I" or pAAV-hSYN-DIO-hM3D(Gq)-mCherry, "PV_E," respectively; n = 12 each; **Supplementary Figure 1**] on the same auditory oddball discrimination task. A subset of PV_I and PV_E rats (n = 8 each) rats were treated with CNO (1 mg/kg, i.p.) 20-30 minutes before each training session, while the rest received saline injections (**Figure 2A**). Saline-treated rats did not differ in their behavioral performance and were thus analyzed together as the trained-control group (T-Ctrl, n = 8). We calculated average performance measures for training bins of 6 sessions per bin, representing approximately one week of training each.

We first calculated the sensitivity index d' to estimate detection of the target stimuli during each session, where d' ≥ 1 indicates successful detection. d' was significantly higher for T-Ctrl and PV_I rats relative to PV_E rats early in training (bin 1, both $p \leq 0.0289$), while the PV_I group performed better than the rest around mid-training (bins 5 and 6, all $p \leq 0.0162$; **Figure 2B**, *left*). When analyzing the number of sessions needed to reach training criterion, we found that PV_I rats required less sessions (21.6 ± 5.8) to reach five consecutive sessions with d' ≥ 1 than either other group (T-Ctrl, 34.5 ± 11.2 sessions, PV_E, 39.5 ± 6.9, both $p \leq 0.037$ relative to PV_I; **Figure 2B**, *right*).

We then analyzed performance as measured by hit rate and FP rate, the two components that determine d' (**Supplementary Figure 2**). Differences in between-group performance around mid-training were significant only in the case of FP-rate, such that PV_I rats had lower FP rates than the T-Ctrl group in training bins 5 and 6 (both $p \leq 0.0367$). These results suggest that, during early stages of training and despite responding to the target tone at the same rate as the other groups, PV_I rats were better at suppressing their response to non-target sounds, which is consistent with previous reports on the role of PV+ interneurons in operant learning (Hu et al., 2014; Caroni, 2015).

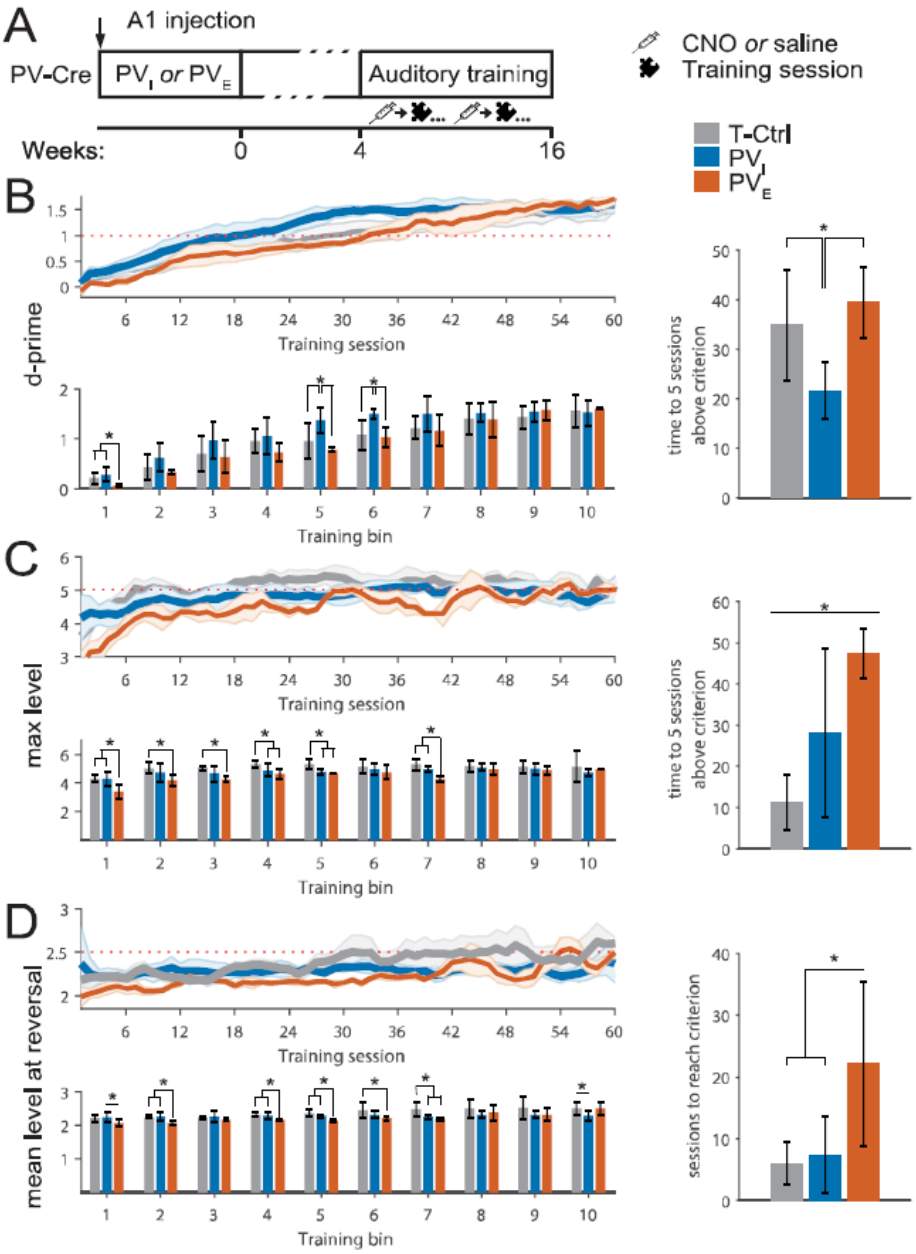

Figure 2. Effects of sustained A1 PV+ cell manipulation on auditory discrimination training. (**A**) *Left*, experimental paradigm. *Right*, the adaptive oddball discrimination training used was the same as described in Figure 1. Repeated measures (rm) ANOVA was used to assess the effect of condition across the training period (F and p values are reported); one-way ANOVA was used to compare the latency (number of sessions) to reach criterion for each behavioral parameter (F and p values are reported); values of follow-up multiple comparisons were corrected with Tukey's post-hoc test. (**B**) *Left*, mean d' reached over 10 training bins (6 sessions/bin) in an adaptive oddball discrimination task. The interaction was significant ($F(18,135) = 2.42$, $p = 0.0021$), as were the simple main effects tests for bins 1, 5, and 6 (all $p \leq 0.03$). *Right*, number of sessions needed to reach d' ≥ 1 ($F(2,18) = 4.56$, $p = 0.0249$; difference relative to PV$_I$ group, all $p \leq 0.037$). (**C**) *Left*, mean maximum level reached over 10 training bins. The interaction was significant ($F(18,126) = 2.49$, $p = 0.0172$), as were the simple main effects tests for bins 1-5 and 7 (all p ≤

0.03). *Right*, no. of sessions needed to reach level 5 or higher ($F(2,19) = 48.37$, $p < 0.001$, all pairwise comparisons $p \leq 0.002$). (**D**) *Left*, mean level at reversal over 10 training bins. The interaction was significant ($F(18,126) = 3.37$, $p = 0.002$), as were the simple main effects tests for bins 1, 2, 4-7, and 10 (all $p \leq 0.04$). *Right*, number of sessions needed to reach mean level at reversal ≥ 2.5 ($F(2,20) = 7.64$, $p = 0.004$, PV_E vs T-Ctrl/PV_I, both $p \leq 0.016$). * $p < 0.05$

Dynamic regulation of PV+ cell activity is required for optimal learning

The use of an adaptive task allowed us to compare performance at increasing levels of difficulty. The difference in the number of sessions needed to consistently reach higher difficulty levels (five consecutive sessions with maximal level ≥ 5) was significant for all between-group comparisons, with T-Ctrl rats requiring the fewest number of sessions to reach criterion (T-Ctrl: 11.2 ± 6.6, PV_I: 28.2 ± 9.4, PV_E: 47.5 ± 5.8; all pairwise comparisons $p \leq 0.002$; **Figure 2C**, *right*). In line with these results, the T-Ctrl group reached a daily maximum level that was significantly higher than the PV_E group during early- and mid-training (bins 1-5 and 7; all ≤ 0.0103), and higher than the PV_I group around mid-training (bins 4 and 5; both $p \leq 0.0393$; **Figure 2C**, *left*).

Given that our task had a one-up one-down staircase design, advancing from one level to the next was relatively easy, as it required only one correct (hit) response. Thus, the information conveyed by maximum level alone might not necessarily represent how well a subject performed during any given session. We therefore calculated the mean level at reversal points, a more sensitive measure to assess improvement in perceptual ability as it represents the difficulty level associated with ceiling in performance during any given training session (Levitt, 1971; Voss and Zatorre, 2012). Again, the T-Ctrl group consistently performed better than the PV_E group during early- and mid-training (bins 2 and 4-7, all $p \leq 0.038$), and better than the PV_I group late in training (bins 7 and 10, all $p \leq 0.04$; **Figure 2D**, *left*). As with previous behavioral parameters, we sought to quantify the latency until achieving consistent increased performance for each group. Because mean level at reversals oscillated between 2 and 2.5 for all groups throughout most of the training period and no rats outside the T-Ctrl group reached 5 sessions with values above 2.5, we calculated instead the number of sessions required to reach a mean level at reversals equal or greater than 2.5. Interestingly, the PV_E group needed three times as many sessions (22.1 ± 13.3) than the other groups to reach this criterion (T-Ctrl: 6 ± 3.4, PV_I: 7.3 ± 6.1; both $p \leq 0.016$ relative to PV_E; **Figure 2D**, *right*).

Prolonged PV+ cell manipulation precludes the full expression of adaptive plasticity associated with learning

Due to their extensive dendritic and axonal arborization across cortical layers (Freund and Buzsáki, 1996; Norenberg et al., 2010) and their position relative to pyramidal cells, PV+ cells are capable of inhibiting neurotransmission near the site of action potential initiation (Hu et al., 2014). From this strategic position, PV+ interneurons contribute to recurrent and lateral inhibition (Almeida et al., 2009; Couey et al., 2013), suggesting that they play a major role in shaping cortical receptive fields (Atallah et al., 2012) and modulating neural synchrony (Agetsuma et al., 2018). To examine the effects of concurrent training and PV+ interneuron manipulation on these functional properties, we mapped of a dense sample of neurons covering the whole area of A1 (Voss et al., 2016) in naive and trained rats

Synchronous neural activity facilitates the processing (Cardin et al., 2009) and integration (Sohal et al., 2009) of sensory stimuli, and has been shown to improve with PL (Fuchs et al., 2007). We measured the degree of spontaneous synchronization between A1 single units by calculating cross-correlation functions for single-unit neuron pairs recorded in silence (as previously done in (Thomas et al., 2019)**; Supplementary Figure 3**). We found a significant effect of condition—T-Ctrl, PV_I, PV_E—on neural synchrony, characterized by an increase in the mean peak correlation coefficient in the T-Ctrl group, relative to naive and PV_I subjects (both $p \leq 0.024$; **Figure 3A,B**).

Training was not associated with any major changes in receptive field selectivity as measured by tuning bandwidth 20 dB SPL above threshold (BW20; **Figure 3C**). Although training paradigms involving the detection of a single target tone may lead to increased (Berlau and Weinberger, 2008; Bieszczad and Weinberger, 2010) or decreased frequency selectivity, our findings are in agreement with previous reports of normal BW values following adaptive discrimination training (de Villers-Sidani et al., 2010; Mishra et al., 2014).

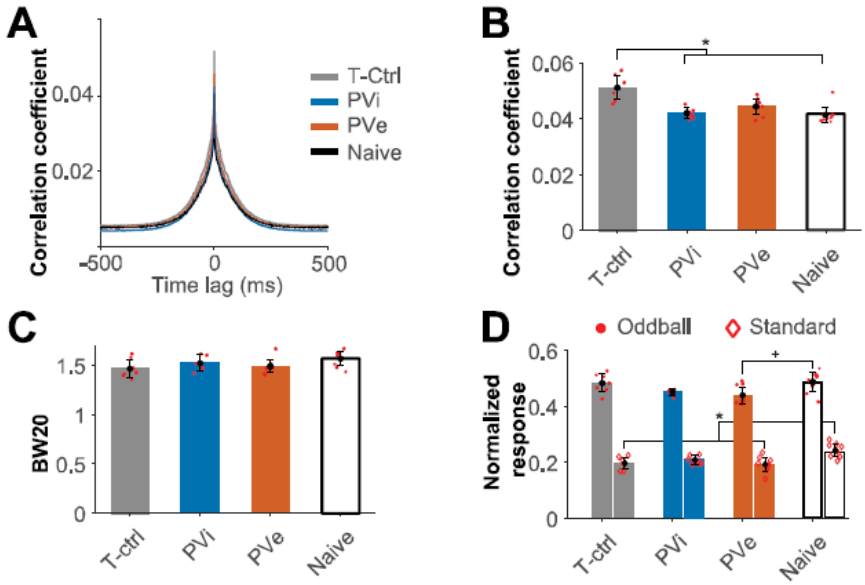

Figure 3. Training-related changes in cortical sensory processing. (**A**) Average cross-correlogram for all pairs < 0.5 mm apart in each experimental condition. Kruskal-Wallis test was used to assess the effect of condition (χ^2 and p values are reported); values of follow-up multiple comparisons were corrected with Tukey's post-hoc test. (**B**) Peak cross-correlation coefficients ($\chi^2(3) = 15.15$, $p = 0.0017$; T-Ctrl vs naive/PVI, both ≤ 0.024, all others, p ≥ 0.18). (**C**) Bandwidth 20 dB SPL above auditory receptive field threshold ($\chi2(3) = 6.28$, $p = 0.09$). (**D**) Stimulus-specific adaptation. Normalized responses to standard (diamonds) and oddball (circles) pure tones presented at 3 p.p.s. Standard, $\chi^2(3) = 11.7$, $p = 0.008$; naive vs T-Ctrl/PV_E, both $p = 0.01$; all others, p ≥ 0.3. Oddball, $\chi^2(3) = 8.75$, $p = 0.032$; PV_E vs naive, $p = 0.07$, all others, p ≥ 0.11. * $p < 0.05$

Neurons along the auditory pathway show a progressively reduced response to repetitive (standard) sounds, which is restored when a deviant stimulus (oddball) is presented (Ulanovsky et al., 2003; Pérez-González and Malmierca, 2014). This form of deviance detection, known as stimulus-specific adaptation (SSA), is involved in the selection of sensory inputs that should be selectively suppressed during training (Froemke et al., 2013). To examine the effect of training on SSA in A1 we used 10-min-long trains of pure tones, which included both standard (80%) and oddball (20%) stimuli (de Villers-Sidani et al., 2010). Training was associated with overall decreased responses to standard tones relative to non-trained subjects, which were significant for the T-Ctrl and PV_E groups (both $p = 0.01$; **Figure 3D**). We also found a significant effect of condition on the response to oddballs following training, but further analysis, controlling for multiple comparisons, revealed only a modest decrease in response to oddballs in the PV_E group, relative to naive rats ($p = 0.07$).

The reduction in responses to both standard and oddball sounds in the PV_E group is consistent with previous research suggesting that PV+ cells contribute to SSA by providing non-specific inhibition (Natan et al., 2015). Taken together, these findings suggest that chronic manipulation of PV+ cell activity precluded the full expression of training-induced adaptive plasticity that is conducive to improved sensory processing (Letzkus et al., 2011; Froemke, 2015; Kuchibhotla et al., 2017). Indeed, among trained rats, only those in the T-Ctrl group exhibited both increased neural synchrony and reduced responses to standard stimuli. We therefore asked whether individual differences in training performance could account for these varied functional outcomes.

Learning progression and its correspondence to sensory processing.

Although considerable research has been devoted to correlating electrophysiological and behavioral outcomes (Blake et al., 2006; Polley et al., 2006; Voss et al., 2016; Irvine, 2018), rather less attention has been paid to the relationship between functional outcomes and longitudinal training performance (Berlau and Weinberger, 2008; Bieszczad and Weinberger, 2010). We considered all of d', hit rate, FP rate, and level data obtained throughout the training period on the one hand; and BW, synchronization, and SSA measurements on the other hand. This approach, however, poses a statistical challenge, as the amount of observations (derived from dozens of training sessions and one recording session) significantly outnumbered that of experimental subjects. To address this issue, we ran a partial-least-squares multivariate analysis (PLS)—a method that has been previously used to examine the relationship between behavioral and functional data (Mirchi et al., 2019)—to obtain individual behavioral (B) and functional (F) scores.

This analysis revealed a statistically significant association between B and F scores ($r = 0.73$; **Figure 4A**). We then investigated the contribution of individual functional parameters to overall B scores. Of the four components derived from PLS (one per functional feature), only the first component was considered, as its variance explained was greater than would be expected by chance (~38% of the covariance between electrophysiology and behavior, $p = 0.05$, all others, covariance ≤ 30%, $p \geq 0.37$; **Supplementary Figure 4**). Whereas all four functional parameters were positively correlated with B scores, response magnitude to oddballs (**Figure 3D**) was the one that most correlated with B scores ($r = 0.70$; **Figure 4B**). In other words, higher F scores (primarily driven by oddball response) were correlated with higher B scores. Note that both scores were, in general, higher for subjects in the T-Ctrl group (**Figure 4A**), which also showed a better overall performance in terms of sessions needed to reach different performance criteria (**Figure 2B-D**).

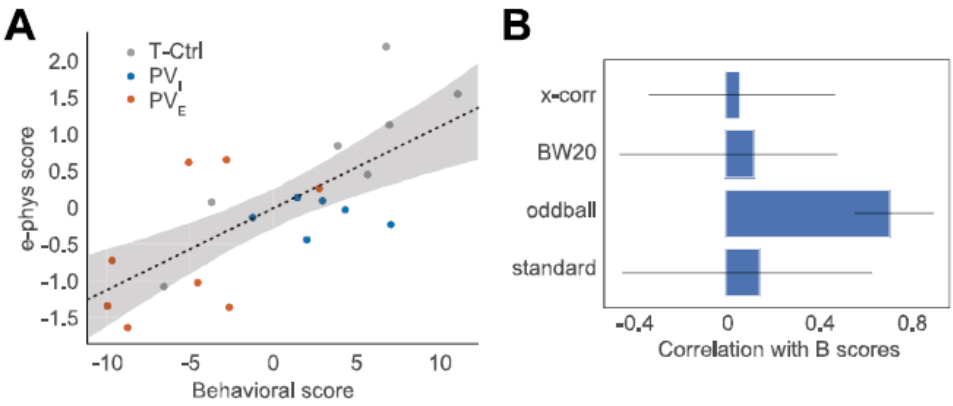

Figure 4. Correlation between behavioral and functional patterns. Individual behavioral and functional scores were estimated for all training sessions and one electrophysiological recording session. (**A**) The scores index the extent to which each subject expressed behavioral and functional patterns. (**B**) Correlations (i.e. loadings) of functional scores with the learning pattern. Error bars represent bootstrap-estimated 95% CIs.

To better understand this relationship, we examined the relative contributions of each behavioral parameter to F scores. This analysis revealed that F scores tended to be positively correlated with the variables maximum level and mean level at reversal throughout the training period, and negatively correlated with FP rate, especially around mid- and late training (**Supplementary Figure 5**). These observations are in agreement with the task structure, whereby enhanced perceptual acuity (oddball detection) and lower FP rates lead to improved performance in terms of levels.

Discussion

To our knowledge, this is the first study of PL that uses Cre-transgenic rats. As rats are the preferred rodent model for the study of the neurophysiological basis of behavior, our findings open the door to future research on the inhibitory regulation of cortical plasticity during learning.

Despite being innovative in terms of both the animal model and analytical approach used, certain shortcomings of our study should be addressed. First, we collected longitudinal data pertaining to behavioral performance but obtained a single readout of neuronal activity. In light of recent

advances in devices for chronic and reliable neurophysiological recordings (Woods et al., 2018), future studies may collect both functional and behavioral data simultaneously over the whole training period to better understand the relationship between neuronal circuit function and performance. Future studies could also help determine whether these measures are correlated with PV expression. Although this matter was beyond the scope of this study, based on previous studies (Wirtshafter and Stratford, 2016; Li et al., 2018; Cisneros-Franco and de Villers-Sidani, 2019), a correspondence between the specific chemogenetic manipulation and PV staining intensity is to be expected. In addition, future studies are needed to determine whether the role of PV-related inhibition on auditory discrimination abilities is lateralized. Whereas previous studies have reported impaired performance in sound localization (Heffner and Heffner, 1990) and discrimination of frequency-modulated tones (Rybalko et al., 2006) following lesions in either left, right, or bilateral auditory cortex, more recent investigations suggest that the processing of physiologically-relevant stimuli might indeed be lateralized (Valtcheva and Froemke, 2019). Given that evidence to date regarding the lateralization of auditory discrimination abilities remains inconclusive, and the more general focus of the present investigation, we decided to perform bilateral virus injections.

Our results suggest that A1 PV+ interneurons bidirectionally regulate plasticity during PL, as has been shown in other cortical areas (Letzkus et al., 2011; Wolff et al., 2014; Caroni, 2015). This possibility is in agreement with the biphasic model of skill learning described by Vinogradov et al. (2012). In this model, *Phase 1* is characterized by rapid improvements in behavior, which have been associated with downregulation of cortical inhibition in animal models (Sale et al., 2010) and in humans (Kolasinski et al., 2019). In light of previous findings that transient specific manipulation of PV+ interneurons affects the initial rate of learning (Letzkus et al., 2011; Donato et al., 2013; Wolff et al., 2014), our results add to the mounting evidence indicating that suppression of PV+ cells is necessary for the initiation of learning (Hu et al., 2014).

Phase 2, in contrast, is characterized by modest behavioral gains coupled with massive reorganization of task-specific representations in the brain (Kleim et al., 2004). In human motor cortex, although this reorganization was not evident until after four weeks of training, it persisted for over 20 weeks without further training (Karni et al., 1995). How do these functional changes relate to PV-inhibition? Evidence from two independent areas of research may contribute to answer this question. First, expertise at late stages—and after the completion—of training is associated with a shift to a ‘high-PV network configuration’ (Caroni, 2015), which is in agreement with reports that activation of PV+ cells improves perception (Lee et al., 2012). Second, although

Phase 2 is characterized by slower learning in healthy subjects, this slowdown is even more pronounced in impaired neural systems, such as in autism (Marín, 2012), aging (de Villers-Sidani et al., 2010), and following noise exposure (Zhou and Merzenich, 2012). As all these conditions are also associated with altered E/I balance and reduced PV expression, it is likely that an increase in PV+ cell function is necessary for late improvements in accuracy and for the consolidation of learning (**Figure 5**).

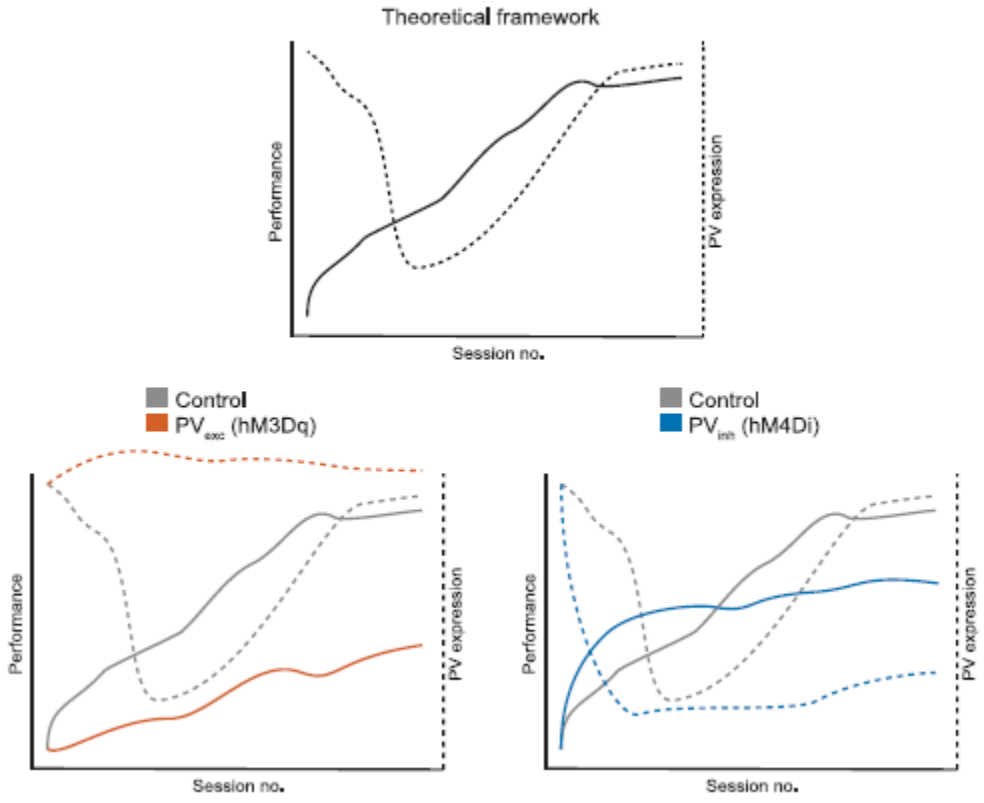

Figure 5. Hypothesized role of PV+ interneurons in perceptual learning. *Top*, trajectory of PV-inhibition and behavioral performance during learning. *Bottom*, hypothesized effects of chronic chemogenetic manipulation of PV+ cells.

Finally, given the role of PV+ cells in learning (Letzkus et al., 2011; Wolff et al., 2014; Caroni, 2015), further behavioural studies involving chronic manipulation of their activity are warranted. These studies may also explore the effects of chronic PV+ cell manipulation on different regulatory elements of plasticity, including other interneuron subtypes and the neuromodulatory systems

that control attention and motivation (Froemke, 2015; Kuchibhotla et al., 2017; Voss et al., 2017). These will improve our understanding of adult plasticity and help devise better rehabilitation strategies for patients with perceptual and cognitive impairments (Kantrowitz et al., 2018; Thomas et al., 2018).

Methods

Experimental subjects

Adult PV-Cre rats were raised from commercially obtained breeding pairs (Horizon Discovery). Animals were housed on a 12-h light/12-h dark cycle and provided with ad libitum food and water unless otherwise noted. All procedures were approved by the local Animal Care Committee and follow the guidelines of the Canadian Council on Animal Care.

Stereotaxic injections

Under isoflurane anesthesia, unilateral virus injections were performed four weeks before the beginning of chemogenetic experiments, as reported previously with Cre transgenic rats(Ferenczi et al., 2016). After exposing the skull, craniotomies were made above left A1 using stereotaxic coordinates(Paxinos and Watson, 2013): bregma AP, -4.5mm; ML, -7mm; DV, 4.5mm. Following dura removal with a 27-G needle, 1 μL virus (pAAV-hSYN-DIO-hM4D(Gi)-mCherry or pAAV-hSYN-DIO-hM3D(Gq)-mCherry was injected using a precision syringe pump (PHD Ultra 4400, Harvard Apparatus, Holliston, MA) at a rate of 0.05 μL/min. The surgical wound was sutured and cleaned, the rat was monitored until full recovery and medicated with antibiotic (enrofloxacin, 10 mg/kg, s.c.) and analgesic (carprofen, 5 mg/kg, s.c.) for at least 3 days (Wirtshafter and Stratford, 2016; Li et al., 2018).

Behavioral training

The rats' behavior was shaped in three phases. During phase A, rats were trained to make a nose poke response to obtain a food reward. During phase B, rats were trained to make a nose poke only after presentation of an auditory stimulus (single 7-kHz tone pip at 60 dB SPL). During phase C, the actual training program, rats were trained at level 1 to make a nose poke only for the target stimulus (containing an oddball frequency) and not for a foil stimulus with six identical tones of frequency identical to the standard frequency (7-kHz). The tones were presented at 60 dB SPL.

Every training session started at level 1. The level was increased after one correct target identification and decreased after a response to a non-target (i.e., false-positive) or miss (one up/one-down). The task difficulty was progressively increased by reducing in exponential steps the frequency difference between standards and oddballs from 0.5 octaves (level 1) to 0.02 octaves (level 6).

Training was performed in an operant training chamber contained within a sound-attenuated chamber. Psychometric functions and stimulus target recognition thresholds were calculated for each training session by plotting the percentage of 'go' responses as a function of the total number of target stimuli (i.e., hit ratio) and the percentage of false positives as a function of the total number of foils (i.e., false-positive ratio). Learning curves were reconstructed by plotting maximal level reached over successive days of training.

A single behavioral trial was defined as the length of time between the onsets of two successive tone trains. The intertrial interval was selected at random from a range of 4 to 6 s. A rat's behavioral state at any point in time was classified as either "go" or "no-go." Rats were in the "go" state when the photobeam was interrupted. All other states were considered "no-go." For a given trial, the rat could elicit one of five reinforcement states. The first four states are given by the combinations of responses (go or no-go) and stimulus properties (target or non-target; **Figure 1A**). Go responses within 3 s of a target were scored as a hit; a failure to respond within this time window was scored as a miss. A go response within 3 s of a non-target stimulus was scored as a false positive. The absence of a response was scored as a withhold. A hit triggered the delivery of a food pellet. A miss or false positive initiated a 5 s "time-out" period during which time no stimuli were presented. A withhold did not produce a reward or a time out.

PV cell manipulation during training

We treated PV_I and PV_E rats with i.p. clozapine-N-oxide (CNO, 1 mg/kg) or vehicle (NaCl, 1 mL/kg) at least 20 minutes before each training session. Earlier studies of DREADDs typically contrasted the effect of CNO administration between subjects that were transfected with either a DREADD or a reporter only (Armbruster et al., 2007; Vardy et al., 2015). Recent findings, however, call into question the need to use CNO treatment as a negative control in chemogenetic experiments. First, CNO itself has no known biological effect and does not cross the blood-brain barrier (Gomez et al., 2017). Indeed, the effects of CNO treatment in DREADD-transfected subjects are the result of systemic conversion to clozapine (Gomez et al., 2017), which at low

CNO doses (1 mg/kg) binds almost exclusively to DREADDs and has no known electrophysiological or behavioral effects (MacLaren et al., 2016). These reasons support the increasing use of vehicle treatment instead of CNO in control subjects (e.g., (Farzi et al., 2018; Zhou et al., 2018), the approach we chose for the present study.

Immunohistochemistry.

Immediately following the end of experiments, rats received a high dose of ketamine/xylazine/acepromazine (130/26/3 mg/kg i.p.) and were perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) at pH 7.2. Expression of markers of interest were examined by fluorescence immunohistochemistry using standard methods (Ouellet and de Villers-Sidani, 2014).

Immediately after perfusion, rat brains were removed and placed in the same fixative overnight for further fixation and then transferred to a 30% sucrose solution, snap-frozen, and stored at -80°C until sectioning. Fixed material was cut in the coronal plane along the tonotopic axis of A1 on a freezing microtome at 40 µm. Tissue was incubated overnight at 4°C in either monoclonal or polyclonal antisera (for anti-GABA: Sigma #A2052, dilution 1:5000; for anti-PV: Sigma #P3088, dilution 1:10 000; for mCherry: ThermoFisher #M11217, dilution 1:2000). Tissue samples were always processed in pairs during immunostaining procedures to limit variables relative to antibody penetration, incubation time, and post-sectioning age/condition of tissue. A Zeiss LSM 710 confocal microscope was used to assess fluorescence in the immunostained sections. Quantification of PV+ cell optical density was performed in Image J and MetaMorph imaging software (Molecular Devices Systems, Toronto, ON), respectively. Digital images of A1 cortical sections were taken with a 40x objective (Zeiss LSM 710). All quantification was assessed in 300-400 µm wide A1 sectors (rostral, middle, caudal) extending from layer 1 to the underlying white matter by an experimenter blind to the age of the animals. PV+ cells were classified into four subclasses according to staining intensity (a.u.) as follows: low-PV, 0-0.4 x 10^3; intermediate low-PV, 0.4-0.8 x 10^3; intermediate high-PV, 0.8-1.2 x 10^3; high-PV, >1.2 x 10^3.

Electrophysiology

Rats were pre-medicated with dexamethasone (0.2 mg/kg) to minimize brain edema. They were anesthetized with ketamine/xylazine/acepromazine (65/13/1.5 mg/kg, i.p.) followed by a continuous delivery of isoflurane 1% in oxygen delivered via endotracheal intubation and mechanical ventilation. Vital signs were monitored using a MouseOx device (Starr Life Sciences,

Holliston, MA). Body temperature was monitored with a rectal probe and maintained at 37 °C with a homeothermic blanket system. The rats were held by the orbits in a custom designed head holder leaving the ears unobstructed. The cisterna magna was drained of cerebrospinal fluid to further minimize brain edema. The left temporalis muscle was reflected, A1 was exposed and the dura was resected. The cortex was maintained under a thin layer of silicone oil to prevent desiccation.

Cortical responses were recorded with 64 channel tungsten microelectrode arrays (Neuronexus, Ann Arbor, MI). The microelectrode array was lowered orthogonally into the cortex to a depth of 470-600 µm (layers 4/5) where vigorous stimulus-driven responses were obtained. The extracellular neural action potentials were amplified, filtered (0.3-5 kHz), sorted, and monitored on-line. Acoustic stimuli were generated using TDT System III (Tucker-Davis Technologies, TDT, Alachua, FL) and delivered in a free field manner to the right ear through a calibrated speaker (TDT). A software package (OpenEx; TDT) was used to generate acoustic stimuli, monitor cortical response properties on-line, and store data for off-line analysis. The evoked spikes of a single neuron or a small cluster of neurons were collected at each site.

Frequency-intensity receptive fields were reconstructed by presenting pure tones of 63 frequencies (1-48 kHz; 0.1 octave increments; 25 ms duration; 5 ms ramps) at 8 sound intensities (0-70 dB SPL in 10 dB increments) in a free-field manner to the right ear at a rate of one stimulus per second. The characteristic frequency (CF) of a cortical site was defined as the frequency at the tip of the V-shaped tuning curve. For flat-peaked tuning curves, the CF was defined as the midpoint of the plateau at threshold. For tuning curves with multiple peaks, the CF was defined as the frequency at the most sensitive tip (i.e., with lowest threshold). The CF and threshold were determined using an automated routine developed in the MATLAB environment (The MathWorks Inc., Natick, MA). To generate A1 maps, Voronoi tessellation (a MATLAB routine; The MathWorks Inc.) was performed to create tessellated polygons with electrode penetration sites at their centers. Each polygon was assigned the characteristics of the corresponding penetration site. In this way, every point on the surface of A1 was linked to the characteristics experimentally derived from its closest sampled cortical site. A1 was identified within auditory cortex based on its rostral-to-caudal tonotopy, reliable short-latency tone-evoked neuronal responses, and relatively sharp V-shaped receptive fields (Polley et al., 2007). To examine A1 map plasticity, we compared the percentage of A1 sites with CFs in 10 bins (width = ½ octave; first and last bins = 1 octave) spanning the spectrum of presented tones.

Statistical analysis.

Analyses were performed using Matlab 2017b. For normally distributed data, ANOVA or repeated-measures ANOVA were used. When data were not normally distributed, nonparametric tests (Kruskal-Wallis) were used. All reported p values were corrected for multiple comparisons with Tukey-Kramer test. PLS analysis was performed as described by (Mirchi et al., 2019), and the code was deposited in an online public repository (Cisneros-Franco, 2019).

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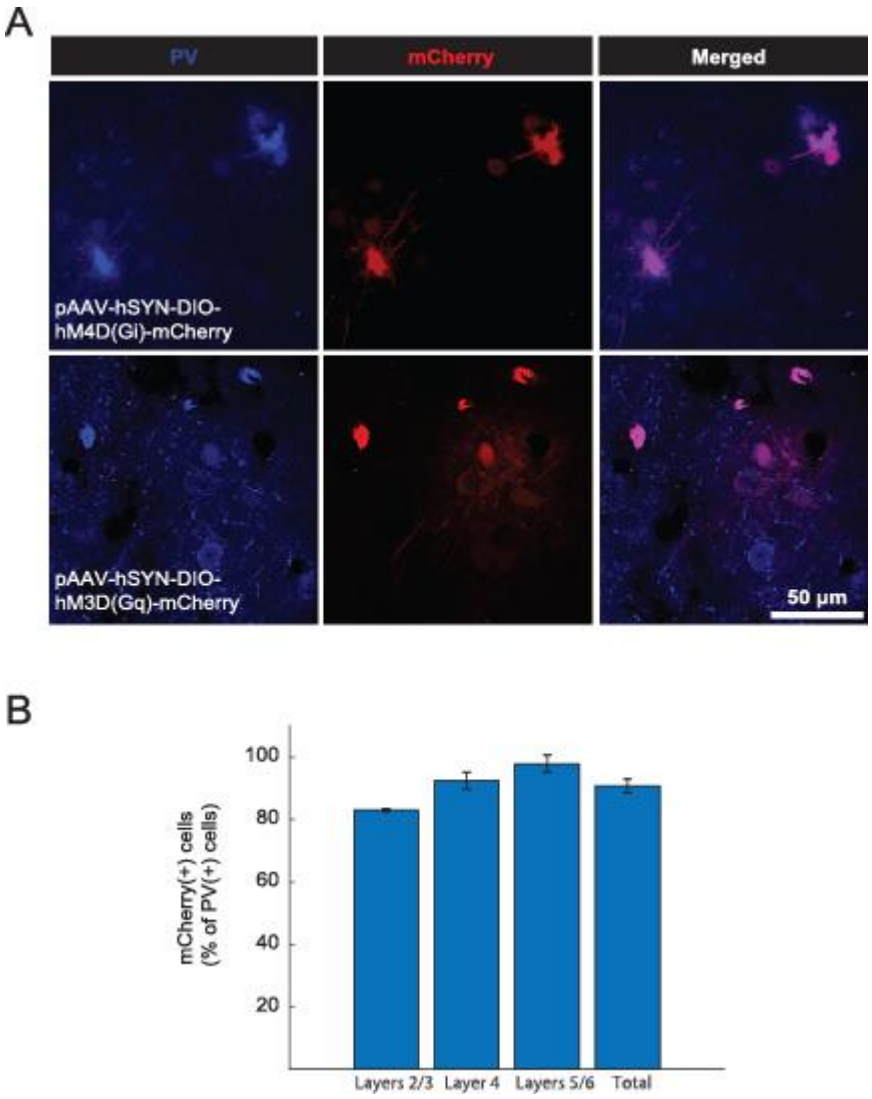
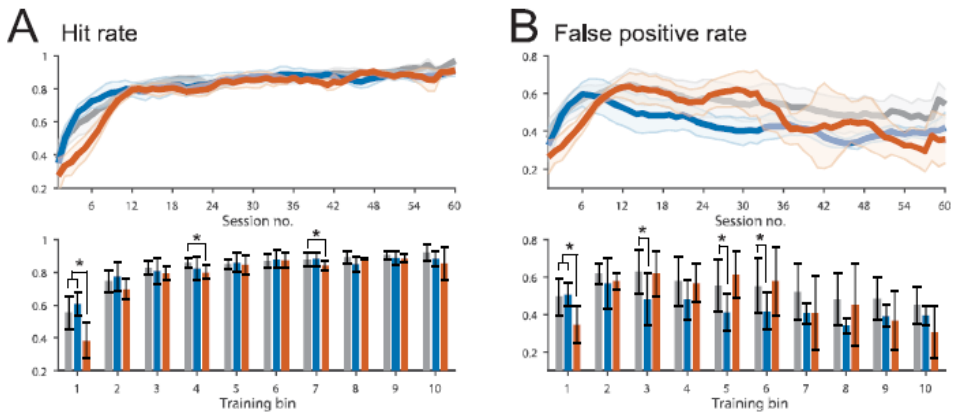
Author contributions

Conceptualization: J.M.C.-F. and E.d.V.-S.; **Methodology**: J.M.C.-F., M.E.T., and L.O.; **Formal analysis**: J.M.C.-F. and I.R.; **Investigation**: J.M.C.-F., M.E.T, I.R., C.L., and L.O.; **Writing – original draft preparation**: J.M.C.-F. and I.R.; **Writing – review and editing**: J.M.C.-F., M.E.T. and E.d.V.-S.; **Visualization**: J.M.C.-F.; **Supervision**: E.d.V.-S.; **Funding acquisition**: E.d.V.-S.

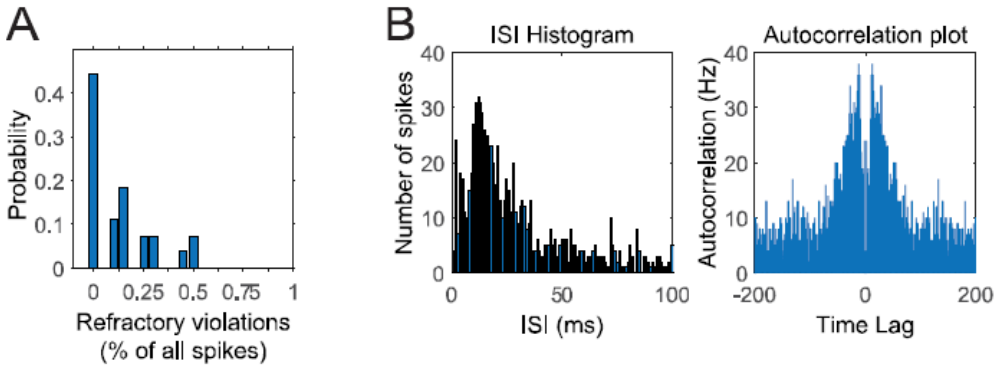
Supplementary information



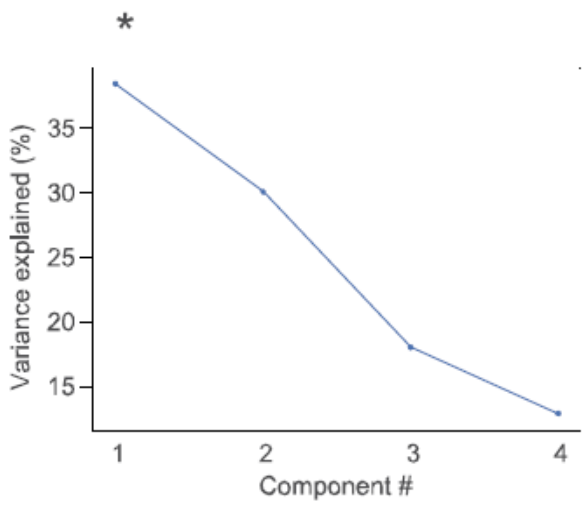
Supplementary Figure 1. Effectiveness of DREADD transfection onto PV+ cells. (**A**) Example of high-power microphotographs of section A1 sections stained for PV and mCherry. (**B**) Overall and layer-specific proportion of PV(+)/mCherry(+) cells.



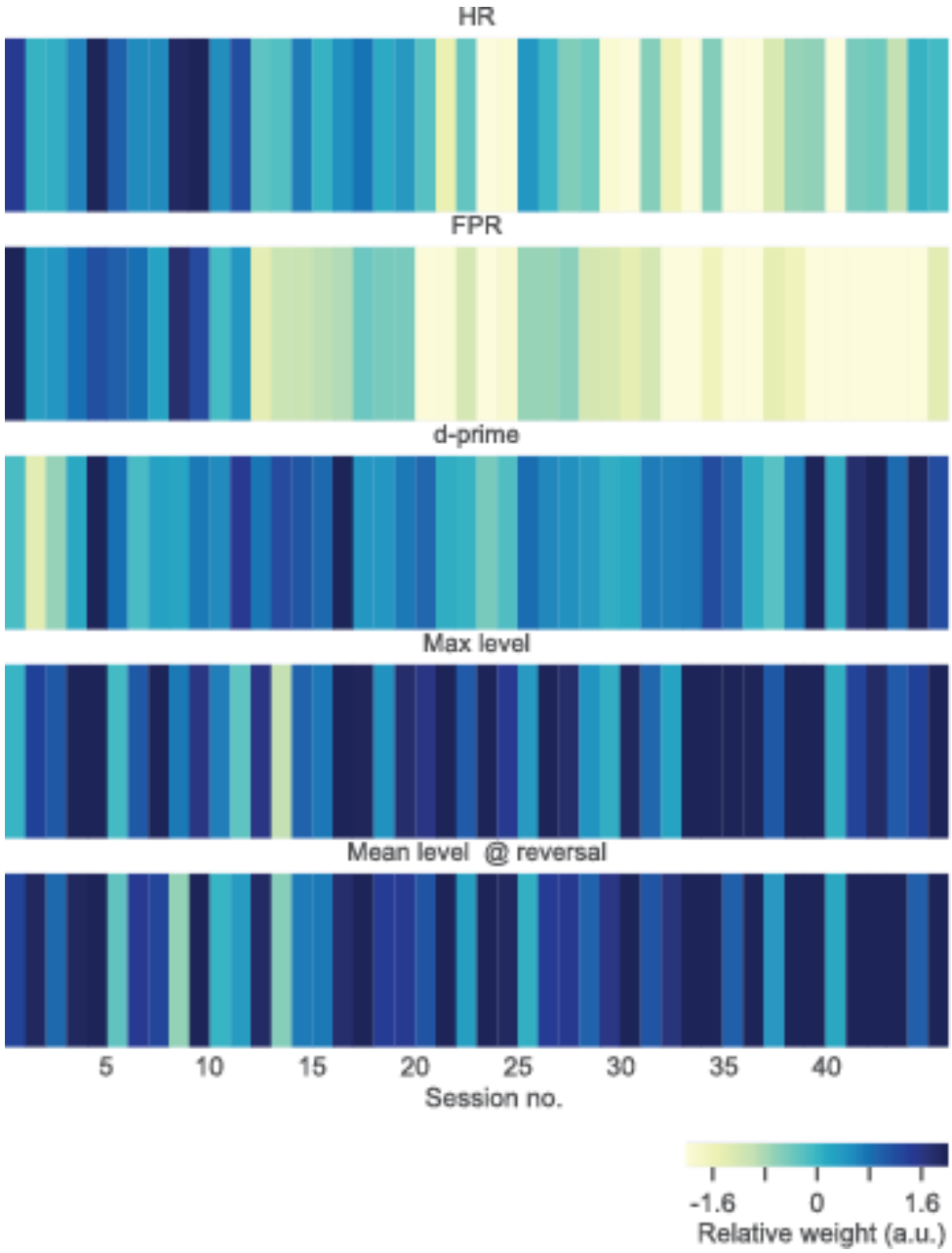
Supplementary Figure 2. Earlier improvement in false-positive rate accounts for accelerated procedural learning observed in the PV_I group. (**A**) Hit rate and (**B**) false-positive rate for all experimental groups. Main effects of group and group x bin interaction were all significant for both parameters (hit-rate: group, $F(2,15) = 29.02$, interaction, $F(9,18) = 4,69$; FP-rate: group, $F(2,15) = 5.44$, interaction, $F(9,18) = 3.01$; all $p \leq 0.0167$, two-way rmANOVA). FP-rate was lower for the PV_I group, relative to the T-Ctrl group for bins 3, 5, and 6 (all $p \leq 0.0367$, with Tukey-Kramer test).



Supplementary Figure 3. Quality assessment for spike sorting. (**A**) Histogram of the fraction of refractory period violations out of all spikes for 491 identified clusters for analyses. (**B**) Examples of histograms for an isolated unit of interspike interval (ISI) times (left) and autocorrelation functions (right). Error bars represent 95% confidence intervals. Number of animals and recording positions per group: Naive, 8, 16; T-Ctrl, 7, 14; PV_I, 6, 12; PV_E, 8, 16.



Supplementary Figure 4. Effect size (variance explained) for each PLS-derived functional component. Permutation sampling was used to perform null-hypothesis significance testing in order to examine the extent to which the variance explained by each component is greater than would be expected by chance. * $p \leq 0.05$



Supplementary Figure 5. Contribution of each behavioral measure to F scores. Functional connections are weighted by bootstrap ratios, a measure of reliability.

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