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Response learning stimulates dendritic spine growth on dorsal striatal medium spiny neurons

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ABSTRACT

Increases in the number and/or the size of dendritic spines, sites of excitatory synapses, have been linked to different types of learning as well as synaptic plasticity in several brain regions, including the hippocampus, sensory cortex, motor cortex, and cerebellum. By contrast, a previous study reported that training on a maze task requiring the dorsal striatum has no effect on medium spiny neuron dendritic spines in this area. These findings might suggest brain region-specific differences in levels of plasticity as well as different cellular processes underlying different types of learning. No previous studies have investigated whether dendritic spine density changes may be localized to specific subpopulations of medium spiny neurons, nor have they examined dendritic spines in rats trained on a dorsolateral striatum-dependent maze task in comparison to rats exposed to the same type of maze in the absence of training. To address these questions further, we labeled medium spiny neurons with the lipophilic dye DiI and stained for the protein product of immediate early gene zif 268, an indirect marker of neuronal activation, in both trained and untrained groups. We found a small but significant increase in dendritic spine density on medium spiny neurons of the dorsolateral striatum after short-term intensive training, along with robust increases in the density of spines with mushroom morphology coincident with reductions in the density of spines with thin morphology. However, these results were not associated with zif 268 expression. Our findings suggest that short-term intensive training on a dorsolateral striatum-dependent maze task induces rapid increases in dendritic spine density and maturation on medium spiny neurons of the dorsolateral striatum, an effect which may contribute to early acquisition of the learned response in maze training.

1. Introduction

A large body of literature has linked several types of learning and memory to changes in dendritic spines, primary sites of excitatory synapses (reviewed in Gipson & Olive, 2017; Bailey, Kandel, & Harris, 2015; Murakoshi & Yasuda, 2012; Yuste, 2011). Synaptic plasticity at the level of the dendritic spine is generally accepted as an important mechanism underlying learning (reviewed in Feldman, 2009; Segal, 2001). Learning and synaptic plasticity have also been associated with structural changes in dendritic spines. Increases in the number of dendritic spines have been reported in the hippocampus in response to spatial navigation learning (Mahmmoud et al., 2015; Moser, Trommald, & Andersen, 1994) and trace or contextual classical conditioning (Leuner, Falduto, & Shors, 2003; Restivo, Vetere, Bontempi, & Ammassari-Teule, 2009), in the motor cortex and cerebellum both in response to acrobatic training/motor learning (Fu, Yu, Lu, & Zuo, 2012; González-Tapia, Velázquez-Zamora, Olvera-Cortés, & González-Burgos, 2015; Ma et al., 2016; Nishiyama, Colonna, Shen, Carrillo, & Nishiyama, 2014), and in the somatosensory cortex in response to sensory learning (Jasinska et al., 2016; Kuhlman, O'Connor, Fox, & Svoboda, 2014). In some cases, studies have also demonstrated that synaptic plasticity (LTP) induces dendritic spine increases in size and number (Park et al., 2006; Yuste & Bonhoeffer, 2001). Additionally, dendritic spine elimination has been shown to promote learning in the hippocampus during contextual fear conditioning (Sanders, Cowansage, Baumgärtel, & Mayford, 2012) and prelimbic cortex during action-outcome learning (Swanson, DePoy, & Gourley, 2017). Taken together, these findings suggest that learning induces change in dendritic spines in almost all systems examined. Evidence has further suggested that the increase in dendritic spine number may be causally linked to task acquisition (Liston et al., 2013) and memory consolidation (Vetere et al., 2003), suggesting that this growth may be an essential aspect of the cellular processes underlying the establishment and potentially, the maintenance of internal representations.

Habit, or response, learning has been linked to the dorsal striatum (Packard & Knowlton, 2002). The only study thus far to examine the

Abbreviations: PBS, phosphate buffered saline; PFA, paraformaldehyde; VTE, vicarious trial and error; DMS, dorsomedial striatum; DLS, dorsolateral striatum

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influence of response learning on structural plasticity in the dorsal striatum reported no change in dendritic spine density or spine size on medium spiny neurons (Hawes et al., 2015). Medium spiny neurons receive specific excitatory inputs from extra-striatal regions, including the neocortex and the ventral tegmental area, onto dendritic spines (Kötter, 1994). The lack of learning-induced change in spine density on medium spiny neurons raises the possibility that these excitatory synapses are less structurally plastic than those in other brain regions and/or that they utilize other cellular processes to acquire response learning associations. It is also worth noting that the striatum is an unusual brain region in that the overwhelming majority of its neurons are inhibitory. Studies have indicated that approximately 99% of neurons in the striatum are inhibitory, with 95% comprising medium spiny neurons and the remainder inhibitory interneurons (Chang, Wilson, & Kitai, 1982; Lim, Kang, & McGehee, 2014). By contrast, only 10–15% of neurons in the hippocampus and 20–30% in the neocortex are inhibitory (Markram et al., 2004; Pelkey et al., 2017), while an even smaller percentage of neurons in the cerebellar cortex, where excitatory granule cells vastly outnumber the other neuron types, are inhibitory (Llinas & Sotelo, 1992). The dramatic difference in the ratio of inhibitory to excitatory neurons in the striatum compared to hippocampus, neocortex, and cerebellar cortex raises the possibility that learning exerts a fundamentally different influence on the striatum in contrast to these other brain regions.

Although medium spiny neurons are so named because of their morphological features, not all of these cells receive the same inputs nor are they functionally homogeneous. Thus, we reinvestigated the question of whether medium spiny neurons undergo dendritic spine growth and/or morphological changes by identifying subpopulations of these neurons based on expression or lack thereof of the protein products of immediate early gene zif 268, an indirect marker of neuronal activation, following early intensive training on a response learning paradigm. Using diolistic (DiI) labeling of medium spiny neurons, here we show an increase in dendritic spine density, more specifically an increase in spines with mushroom morphologies, in the dorsolateral striatum-dependent maze-trained group, an effect that appears to be specific to the dorsolateral striatum, but not to zif 268 labeled neurons.

2. Materials and methods

2.1. Animals and food deprivation

All animal procedures were performed in accordance with the Princeton University Institutional Animal Care and Use Committee regulations and conformed to the National Research Council Guide for the Care and Use of Laboratory Animals (2011). Adult male Sprague-Dawley rats (8–10 weeks-old, Taconic Farms, Inc.) were pair-housed in standard cages under a reverse 12-hour light–dark schedule (lights off at 0700). Rats were habituated to experimenter handling by passive holding once a day for 7 days, during which time they began food restriction. To motivate food reinforcement seeking, rats were food-restricted 5 days prior to behavioral training to maintain 85% body weight and given Kellogg Froot-Loop halves in their home cage in order to habituate to the novel food prior to training.

2.2. Response learning paradigm

To assess the effects of early training acquisition on a response training task, we used a plus maze paradigm (adapted from Chang & Gold, 2003) which requires a specific motor response (right or left-hand turn) while traversing a maze for food reinforcement. This task involved 3 days of maze exposure (see Fig. 1a). We used this paradigm to capture early response acquisition within single sessions of training and testing.

The maze was enclosed in opaque curtains to minimize reliance on extra-maze visual cues and all maze exposure was conducted in the dark under red light illumination. Maze habituation, training, and testing were video recorded by a ceiling-mounted camera centered over the maze. Identical food cups were placed at the ends of all open arms. During habituation, Plexiglas barricades were used to block entry to 4 of 8 arms on an 8-arm radial maze to construct a plus-maze. During training, barricades were used to block entry to 5 of 8 arms on the maze to construct a T-maze. During testing, barricades were used to block entry to 4 of 8 arms on the maze (differing from the arms during habituation) to construct a plus-maze (see Fig. 1a).

2.2.1. Controls and experimental design

Maze-enriched controls, which we will proceed to refer to as maze controls, used the same maze configurations as described above with a variable reinforcement contingency to promote non-strategic navigating, but with the same amount of exposure to the maze as their response trained counterparts, which we will proceed to refer to as response learners. We conducted this experiment twice. In the first experiment, we searched for evidence of dendritic spine density differences on dorsomedial and dorsolateral striatum medium spiny neurons between response learners and maze controls. In the second experiment, we examined spine density, morphology, and size on medium spiny neurons while considering brain side, lateralized to the trained response, and training-induced expression of the protein products of the immediate early gene zif 268 in response learners and maze controls. Behavioral manipulations were identical for both experiments.

2.2.2. Habituation

Both response learners and maze controls were given 4 trials to explore the plus maze for 180 s per trial. Start arm for each trial was randomized and non-repeating. After the completion of a trial, rats were placed in their home cage behind the start arm for a 30 s intertrial interval (ITI). Trained response (left or right-turn for reinforcement) was determined based on the initial turn, e.g., if on the first trial a rat turned right, then the assigned reinforced response for training and testing would be a left-turn response.

2.2.3. Training

Response learners were given a maximum of 70 trials on training day to reach criterion with a maximum time of 120 s per trial. Start arm for each trial was pseudo-randomized, where arms were randomized within blocks of 4 trials. A trial was complete once reinforcement was retrieved (made a correct arm entry), made an incorrect arm entry, or timed out. If an incorrect arm entry was made during the first 4 trials, rats were allowed to trace back to the correct arm. After the completion of a trial, rats were placed in their home cage behind the start arm for a 30 s ITI. Arms of the maze were rotated 90° counterclockwise after 3 correct choices in a row. Response learners were required to make the correct response 6 times in a row to reach criterion. Maze controls were yoked to the average number of response training trials and given a maximum of 120 s per trial. Start arm for each trial was pseudo-randomized, where arms were randomized within blocks of 4 trials. Reinforcement schedule and distribution was randomized to prevent any strategy acquisition.

2.2.4. Testing

Response learners were given a maximum of 70 trials on testing day to reach criterion with a maximum time of 120 s per trial. Start arm for each trial was pseudo-randomized, where arms were randomized within blocks of 4 trials. Trials were complete once reinforcement was retrieved (made a correct arm entry), not retrieved (made an incorrect arm entry), or timed out. After the completion of a trial, rats were placed in their home cage behind the start arm for a 30 s ITI. Arms of the maze were rotated 90° counterclockwise after 3 correct choices in a row. Response learners were required to make the correct response 9 out of 10 times to reach criterion. Maze controls were yoked to the average number of response testing trials and given 120 s for the first half of trials and 60 s for the last half of trials. Start arm for each trial

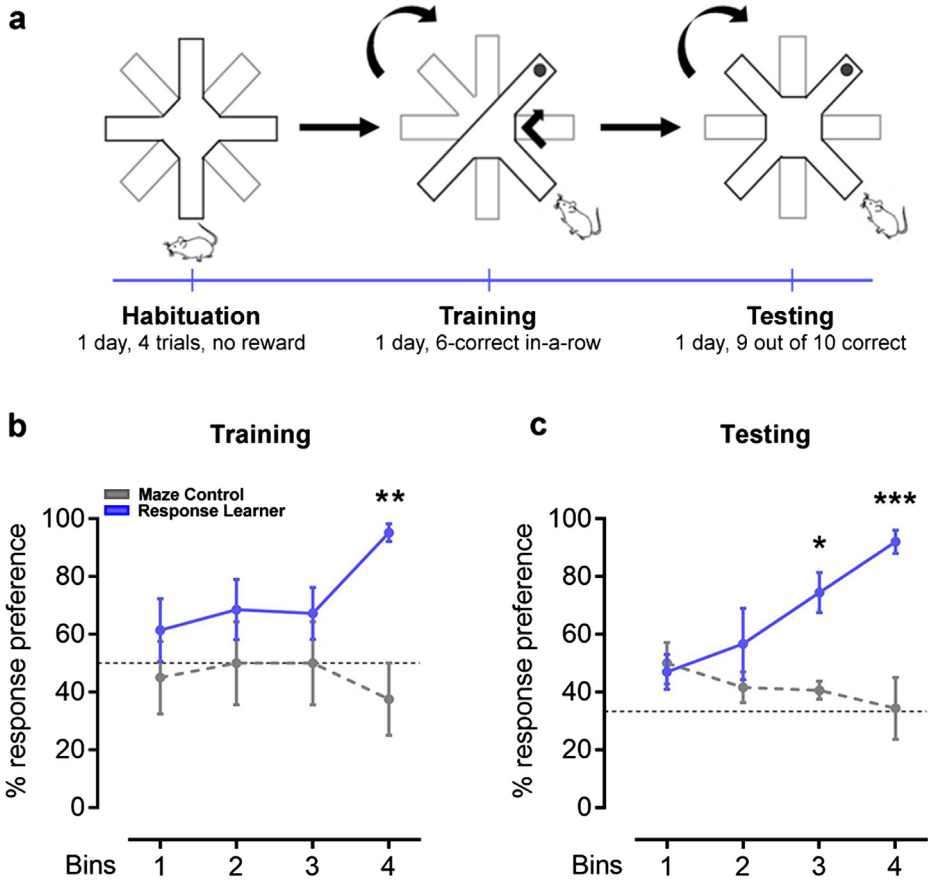

Fig. 1. Short-term training paradigm entrains a response learning strategy and increases response preference above chance levels. (a) Timeline and depiction of early training paradigm for response learners. Black circles depict location of food reinforcer. (b) Line graph of percent response preference during training showing greater preference in response learners (blue) compared to maze controls (grey) during bin 4. Dotted black line depicts chance levels for preference (50%, two-response options). (c) Line graph of percent response preference during testing showing greater preference in response learners (blue) compared to maze controls (grey) during bin 3 and 4. Dotted black line depicts chance levels for preference (33.3%, three-arm options), (n = 4–7) $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

was pseudo-randomized, where arms were randomized in blocks of 4 trials. Reinforcement schedule and distribution were randomized to prevent any strategy acquisition.

2.3. Vicarious trial and error measurement and performance analysis

In the second experiment, vicarious trial and error (VTE) behavior was scored using BIOBSERVE tracking software, where a movement of the head from the direction of one arm to another at a choice point (in most cases the center hub of the maze) was counted as one instance of VTE (Hu & Amsel, 1995). If multiple entries through a choice point were made during a single trial, an average VTE score per entry was calculated for that trial. In both the first and second experiment, response preference was determined manually. For each trial, the initial response made out of the start arm, a right or left-hand turn during training, or the added option to go straight during testing, was recorded. For response learners, preference for the rewarded response was analyzed. For maze controls, preference for a right-hand turn response was analyzed.

2.4. Perfusion

Approximately 1 h after testing, all rats were deeply anesthetized with Euthasol and transcardially perfused with 1.5% paraformaldehyde (PFA) in phosphate buffered saline (PBS), pH 7.5. The brains were dissected and postfixed in 1.5% PFA for 72 h before processing. The post-training perfusion time point was selected to examine dendritic spines shortly after training and for the second experiment involving identification of zif 268 protein-expressing medium spiny neurons, at a post-neuronal activation time point when detection of immediate early gene protein products has been verified (Gill, Bernstein, & Mizumori, 2007; Rogue & Vincendon, 1992).

2.5. DiI labeling and DiI co-labeling with zif 268 immunolabeling

To assess potential changes in dorsal striatum medium spiny neuron dendritic spines, 80 μm coronal sections (1:6) were cut from half brains into a bath of 0.1 M PBS, pH 7.5 using a Vibratome. Individual sections were shot with lipophilic DiI coated Tungsten particles using the BioRad Helios Gene Gun system (BioRad) and incubated for 24 h at 4 °C. Sections were then postfixed in 4% PFA for 1 h at room temperature, washed, and mounted onto suprafrost slides and coverslipped using Vectashield. To assess potential changes in dendritic spines of medium spiny neurons which expressed protein products of the immediate early gene zif 268, tissue was collected as described above. Individual sections were shot with DiI as described above, free-floating sections were rinsed in 0.1 M PBS, pH 7.5, and incubated with 10% normal donkey serum, 0.1 M PBS with 0.01% Triton X-100, and rabbit anti-zif 268 (1:500; Santa Cruz Biotechnology, Cat# sc-189, RRID:AB_2231020), and stored at 4 °C for 24 h. After incubation in primary antisera sections were washed and incubated with Alexa Fluor 488 (1:200; Invitrogen) in the dark for 2 h at room temperature. Sections were then postfixed in 4% PFA for 1 h at room temperature; washed, mounted, and coverslipped as described above. Slides were coded until completion of analysis.

Both hemispheres were examined in response learners due to previous reports of hemispheric differences in neuronal activation as a result of learned motor responding (Cui et al., 2013; Kravitz et al., 2010). Hemispheres were categorized as either contralateral or ipsilateral to the learned motor response (e.g., a right-turn learned motor response = left hemisphere as contralateral and right hemisphere as ipsilateral to the turning response). Maze control hemispheres were neither, and thus were characterized as non-lateral.

2.6. Confocal microscopy

Images from dorsal striatum were taken using a Zeiss confocal microscope (LSM 700; lasers, argon 458/488; HeNe 568). Distinctions between dorsomedial and dorsolateral were based on region depictions by Devan, McDonald, & White, 1999 (medial to bregma, 1.5–2.5 mm; lateral to bregma, 3.0–4.0 mm). Density measurements of cell bodies labeled with mouse monoclonal antibody against NeuN, clone A60 (1:500, Millipore-Sigma Cat# MAB377) co-labeled with zif 268 immunolabeling were acquired using a 20 × 0.75 NA objective from 20 μm image stacks using ImageJ (NIH), where NeuN positive labeled cells and NeuN positive zif 268-positive co-labeled cells were counted exhaustively to obtain the density of neurons that expressed the protein product of zif 268. DiI labeled medium spiny neurons were visualized by using a 63 × 1.40 NA oil objective. Dendritic segments were obtained by imaging representative secondary or tertiary dendritic branches, without overlapping dendrites from other labeled neurons, from 5 different cell bodies, for each brain region from each rat. Double-labeled DiI–zif 268 sections were visualized in the same manner as described for DiI. Because previous studies have shown neuronal activity changes related to brain side in tasks that involve repetitive left or right motor responses (Cui et al., 2013; Kravitz et al., 2010), we performed our analysis on both sides of the brain, separately, for response learners. For maze controls, we counterbalanced left and right hemispheres for analysis. Dendritic segments were obtained by imaging representative secondary and tertiary dendritic branches from a total of 10 medium spiny neurons in the dorsolateral striatum per hemisphere: 5 different cell bodies expressing zif 268 protein and 5 different cell bodies not expressing zif 268 protein, which we will from now on refer to as zif 268-positive and zif 268-negative, respectively. Dendritic segment measurements totaled 50 μm per neuron.

Spines were defined as obvious protrusions from the dendritic shaft with a length greater than 0.2 μm and spine neck width no greater than the dendritic shaft. Spine measurements (length, spine head width, morphology) were analyzed and traced by hand from 5–25 μm image stacks using ImageJ (NIH). Spine morphologies were determined based on previously published characterizations (Lee et al., 2006; Peters & Kaiserman-Abramof, 1970; Yuste & Bonhoeffer, 2004). Spines were classified as (1) stubby if the length was less than 1.0 μm and had no difference in width between the spine head and shaft; (2) mushroom if the length was greater than 0.5 μm and did show a difference in spine head and shaft, where the spine head was much larger (on average > 0.5 μm) than the shaft; (3) thin if the length was less than 2.0 μm and had a small head (on average ≤ 0.5 μm), where the spine head was larger than the shaft; and (4) filopodia if the length was greater than 2.0 μm, width less than 1.0 μm, and lacking an obvious head (see examples in Fig. 6).

2.7. Statistics

Percent response preference data and VTE measures were analyzed using a repeated measures two-way ANOVA (within-subjects) with Sidak post hoc comparisons. Histology data collection and analyses were performed by an experimenter unaware of the animal group. Independent variables for dendritic spine data included: region (dorsolateral or dorsomedial striatum), maze group (maze control or response learner), and hemisphere (non-lateralized, contralateral, or ipsilateral), for DiI-labeled and DiI-double labeled activation (zif 268-positive or zif 268-negative). Both DiI-labeled and DiI-double labeled dendritic spine data were analyzed using a linear mixed effects (lme) model, using the lme4 package (Bates et al., 2014), for comparing maze controls to response learner contralateral and ipsilateral (combined and separately) spine data, within one region. Population design weighted lme modeling was used for DiI-double labeled medium spiny neuron populations, where both zif 268-positive and zif 268-negative medium spiny neuron populations were combined, respective of percent neurons that were zif 268-positive per hemisphere per animal. The lme model was used in place of standard parametric tests because it avoids over-averaging errors in big data sets, weights unequal sample sizes accordingly, is a better statistical model for analyzing between (in our study maze control versus response learners) and within (hemisphere) subject comparisons, and models individual variances. It should be noted that, after computing the entire data set, the LME compares density per rat, not per number of dendritic segments. Unpaired Student's *t*-test were used for comparisons between two groups. Data sets were tested for outliers using a Grubbs' test with alpha = 0.01. GraphPad Prism 7.0 (GraphPad Software) and RStudio (RStudio Team, 2016) were used for statistical analyses.

3. Results

3.1. Response training results in increased response preference with the emergence of stereotyped behavior

Percent response preference data for the first study was binned into 4 quarters of trials and analyzed for both training and testing. On average, response learners took 17 trials to reach training criterion and 34 trials to reach testing criterion. During training, repeated measures two-way ANOVA (within-subjects) of percent response preference across bins showed a significant main effect between response learners and maze controls ($F_{(1, 9)} = 5.806$, $p = 0.0393$), where post hoc analysis showed a significantly greater response preference in response learners (95.23 ± 3.08%), greater than 50% chance levels, compared to maze controls (37.5 ± 12.5%) in bin 4, only ($p = 0.0028$) (Fig. 1b). During testing, repeated measures two-way ANOVA (within-subjects) of percent response preference across bins again showed a significant main effect between response learners and maze controls ($F_{(1, 9)} = 10.01$, $p = 0.0115$) and a significant interaction effect ($F_{(3, 27)} = 6.686$, $p = 0.0016$), where post hoc analysis showed a significant increase in response preference in response learners (92.046 ± 3.99%) compared to maze controls that showed at chance preference (34.38 ± 10.67%) in bin 4 ($p = 0.0001$) and bin 3 (M: 40.63 ± 3.13%; R: 74.47 ± 6.93%; $p = 0.0296$) (Fig. 1c).

3.2. Response training increases the density of dendritic spines on medium spiny neurons of the dorsolateral, but not dorsomedial, striatum

Two days of exposure to the response learning task (one day of training, one day of testing) resulted in a significant increase in the density of dendritic spines on medium spiny neurons of the dorsolateral striatum (confocal images shown in Fig. 2a) compared to maze controls (lme model: β = 1.6293, SE = 0.6003, $p = 0.00915$) (Fig. 2b). By contrast, no significant difference was observed in the density of response learning and maze control dendritic spines on medium spiny neurons of the dorsomedial striatum (lme model: β = −0.1821, SE = 1.2002, $p = 0.883$) (Fig. 2b). No differences were observed in spine size (length or head width) among medium spiny neurons of either brain region (DLS, length: β = −0.01663, SE = 0.05930, $p = 0.786$; DMS, length: β = −0.01131, SE = 0.05823, $p = 0.85$; DLS, width: β = −0.009849, SE = 0.020821, $p = 0.65$; DMS, width: β = −0.01498, SE = 0.03471, $p = 0.676$) (Fig. 2c and d). Because the spine density difference was observed only in the dorsolateral striatum, we confined our subsequent analyses to this brain region.

3.3. Response training results in reduced vicarious trial and error behavior coincident with the emergence of stereotyped behavior

To further investigate the effects from the first study, we tested a second cohort of rats on the response learning task. During training, repeated measures two-way ANOVA (within-subjects) of percent response preference across bins showed a significant main effect between response learners and maze controls ($F_{(1, 17)} = 18.81$, $p = 0.0004$) and

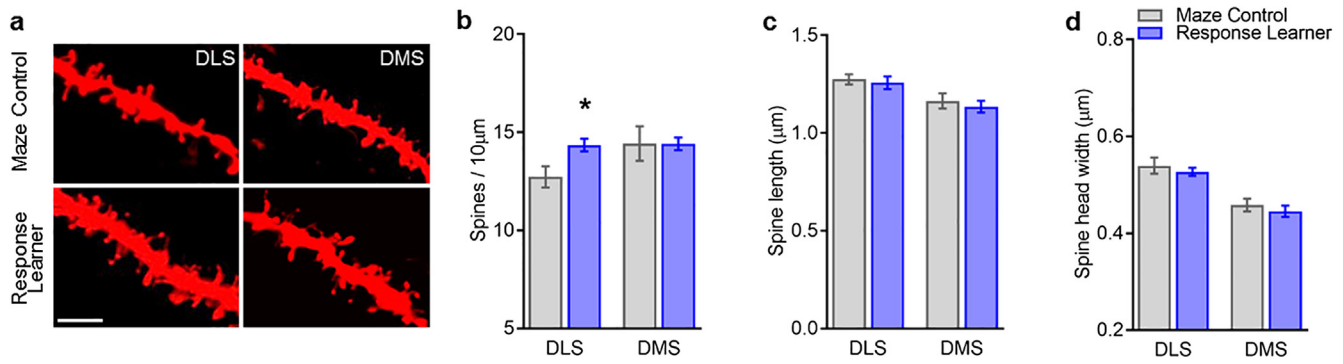

Fig. 2. Response learning increases density of dendritic spines on medium spiny neurons in the dorsolateral striatum during early training. (a) Representative images of medium spiny neuron dendritic segments (DiI, red) in the dorsolateral (left) and dorsomedial (right) striatum of maze control (top) and response learner (bottom) groups (n = 4–7). Scale bar equals 5 µm. (b) Spine density difference between maze controls (grey) and response learners (blue) observed in the dorsolateral striatum only. (c) Spine length does not change with response learning in either dorsolateral or dorsomedial striatum. (d) Spine width does not change with response learning in either dorsolateral or dorsomedial striatum. Box and whisker graphs are plotted from min to max. $^{*}p < 0.05$; DLS, dorsolateral striatum; DMS, dorsomedial striatum. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

a significant interaction effect ($F_{(3, 51)} = 8.662$, $p < 0.0001$), where post hoc analysis showed a significant increase in response preference in response learners (92.57 ± 4.29%) compared to maze controls (33.33 ± 7.22%) at bin 4 ($p < 0.0001$) (Fig. 3a). During testing, repeated measures two-way ANOVA (within-subjects) of percent response preference across bins again showed a significant main effect between response learners and maze controls ($F_{(1, 17)} = 11.6$, $p = 0.0034$), a significant main effect across bins ($F_{(3, 51)} = 12.15$, $p < 0.0001$), and a significant interaction effect ($F_{(3, 51)} = 16.14$, $p < 0.0001$). Post hoc analysis showed a significant increase in response preference in response learners (91.5 ± 2.89%) compared to maze controls (41.67 ± 4.17%) in bin 4 ($p < 0.0001$), in addition to bin 3 ($p = 0.0027$) (Fig. 3b). Coincident with a robust response preference by bin 4, response learners also qualitatively became more stereotyped in the path taken to the food reinforcer by the end of testing (Fig. 3c).

We also analyzed vicarious trial and error (VTE) behavior exhibited in the center of the maze during the task. VTE behavior, which occurs mostly during the early stages of maze learning, has been used as a measure of deliberate processing (Muenzinger & Gentry, 1931; Muenzinger, 1938; Redish, 2016; Tolman, 1938, 1939; van der Meer & Redish, 2010) where a decrease in VTE behavior coincides with response learning. VTE behavior was analyzed in 4 bins of trials for both training and testing for maze control and response learners. During training, repeated measures two-way ANOVA (within-subjects) analysis showed a significant main effect across bins ($F_{(3, 51)} = 4.452$, $p = 0.0075$). In maze controls, VTE behavior increased between bin1 (5.41 ± 0.82) and bin 2 (9.58 ± 2.26) ($p = 0.0202$) and bin 1 and bin 3 (10.01 ± 1.7) ($p = 0.0081$) (Fig. 3d). In response learners, VTE behavior did not significantly change across bins (Fig. 3d). During testing, repeated measures two-way ANOVA (within-subjects) analysis revealed a significant main effect between response learners and maze controls ($F_{(1, 17)} = 6.702$, $p = 0.0191$) and a significant interaction effect ($F_{(3, 51)} = 5.386$, $p = 0.0027$) (Fig. 3e). Post hoc analysis showed response learners exhibited significantly less VTE behavior compared to maze controls during bin 3 (M: 8.81 ± 2.27; R: 3.20 ± 0.76; $p = 0.0363$) and bin 4 (M: 9.11 ± 1.63; R: 1.13 ± 0.25; $p = 0.0012$) (Fig. 3e).

3.4. Response training increases the density of dendritic spines on medium spiny neurons, but is not specific to the zif 268-positive population

To determine whether the increase in dendritic spine density seen in our first experiment examining early training was driven by medium spiny neurons that were activated during maze behavior, we carried out a DiI analysis in conjunction with immunolabeling for the protein product of the immediate early gene zif 268, an indirect marker of neuronal activation (confocal images shown in Fig. 4a). We also investigated whether spine changes were predominantly on one side of the brain or the other during the response learning task (contralateral or ipsilateral to the turning side). First we confirmed that response learning increased the density of zif 268-posiitve NeuN-positive neurons in the dorsolateral striatum (maze control: 7700 ± 906.4 cells/mm^3; response learners: 10,982 ± 926.8 cells/mm^3; $p = 0.0222$), and not in the dorsomedial striatum (maze control: 9341 ± 1757 cells/mm^3, response learners: 8849 ± 1243 cells/mm^3; $p = 0.8222$). Then we analyzed dendritic spines on zif 268-positive and zif 268-negative medium spiny neurons in the dorsolateral striatum (confocal images shown in Fig. 4b). Surprisingly, analyses of spine density on zif 268-positive and zif 268-negative medium spiny neurons, separately, did not reveal any significant spine density differences in either subpopulation (zif 268-positive: β = 0.2708, SE = 0.4292, $p = 0.529$; zif 268-negative: β = 1.0746, SE = 0.7639, $p = 0.165$), nor were differences detected on one side of the brain or the other in either subpopulation (zif 268-positive: β = 0.3819, SE = 0.4393, $p = 0.386$; zif 268-negative: β = −0.5295, SE = 0.7361, $p = 0.475$) (Fig. 4c and g). Again, no differences were observed in spine size (length or head width) among medium spiny neurons of either zif 268-positive or zif-negative (zif 268-positive, length: β = −0.0479, SE = 0.03397, $p = 0.173$; zif 268-negative, length: β = −0.05188, SE = 0.04553, $p = 0.27$; zif 268-positive, width: β = −0.001654, SE = 0.015095, $p = 0.914$; zif 268-negative, width: β = 0.0098196, SE = 0.0138948, $p = 0.483$) (Fig. 4e, f, i and j).

Despite the lack of significant differences in spine density within either of these two subpopulations, both the zif 268-positive and zif 268-negative medium spiny neurons showed significant differences in the density of spines with a mushroom morphology. Zif 268-positive medium spiny neurons showed a hemispheric difference, where the ipsilateral hemisphere of response learners had a greater mushroom spine density compared to maze controls non-lateralized hemisphere (β = 0.6069, SE = 0.2979, $p = 0.0436$; ipsilateral vs. non-lateral: $p = 0.0109$) (Fig. 4d). Zif 268-negative medium spiny neurons showed an overall significant difference in mushroom spine density between response learners and maze controls (β = 1.9748, SE = 0.7079, $p = 0.0147$), where both contralateral and ipsilateral response learner hemispheres had a greater mushroom spine density compared to maze controls non-lateralized hemispheres. Additionally, there was an ipsilateral hemispheric decrease in the density of spines with a thin morphology compared to both response learner contralateral and maze control non-lateralized hemispheres (β = −0.8394, SE = 0.3359, $p = 0.0153$; ipsilateral vs. non-lateral: $p = 0.001717$, ipsilateral vs. contralateral: $p = 0.02491$) (Fig. 4h).

We next investigated the proportion of neurons in the dorsolateral striatum that were zif 268-positive for each individual animal and used

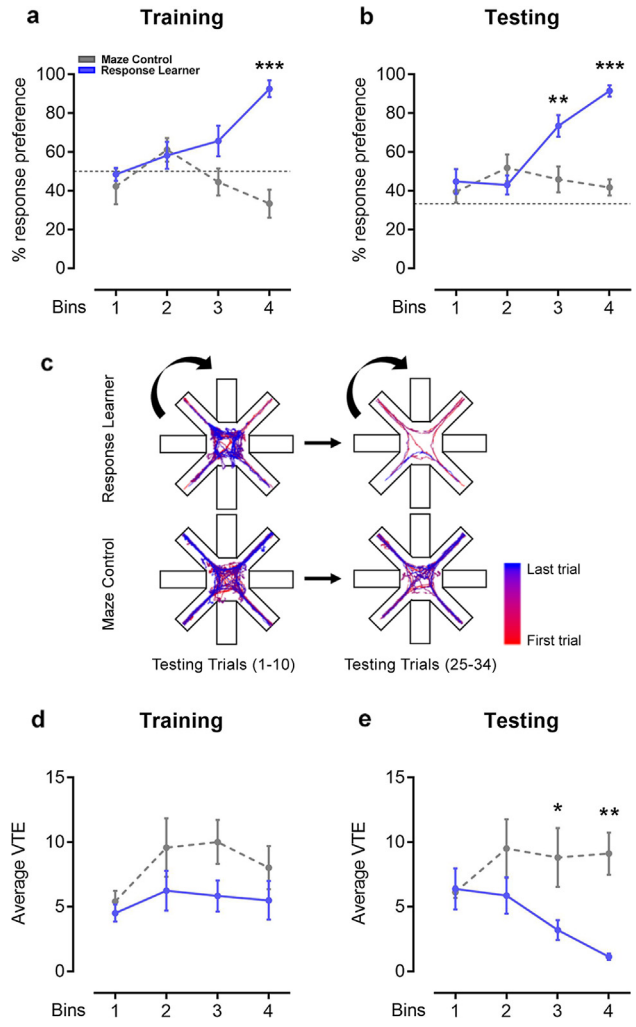

Fig. 3. Response training results in reduced vicarious trial and error behavior coincident with the emergence of stereotyped behavior. (a) Line graph of percent response preference during training showing greater preference in response learners (blue) compared to maze controls (grey) during bin 4. Dotted black line depicts chance levels for preference (50%, two-response options). (b) Line graph of percent response preference during testing showing greater preference in response learners (blue) compared to maze controls (grey) during bin 3 and 4. Dotted black line depicts chance levels for preference (33.3%, three-arm options) (n = 9–10). (c) Representative path traces of response learner (top) and maze control (bottom). Trials 1–10 during testing depicted on the left and trials 25–34 during testing depicted on the right. Red to blue color scale corresponds to successive trials. (d) Average VTE behavior increases in maze controls (grey), only, during training. Average VTE behavior does not change across time in maze controls (grey), but decreases in response learners (blue) during testing. VTE, vicarious trial and error. Testing trial paths shown are taken from one response learner and one maze control. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.0001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

those data to analyze the two subpopulations combined (zif 268-positive and zif 268-negative). Spine density data from this experiment were analyzed using a population-specific weighted approach to better approximate the dorsolateral striatum medium spiny neuron population as a whole and to more accurately reflect the random selection of medium spiny neurons from our first study. The weighted analysis replicated our previous finding of increased dendritic spine density on medium spiny neurons in response learners compared to maze controls ($\beta = 0.8961$, SE = 0.4263, p = 0.0455) (Fig. 5a). We also found an increase in the density of spines with a mushroom morphology in response learners compared to controls ($\beta = 1.24672$, SE = 0.42426, p = 0.00771) and an ipsilateral hemispheric difference in thin spine density, where ipsilateral response learners had fewer thin spines ($\beta = -0.4631$, SE = 0.1816, p = 0.0115; ipsilateral vs. non-lateral: $p = 0.001058$, ipsilateral vs. contralateral: $p = 0.021585$) (Fig. 5b). By contrast, no differences were observed in the density of spines between response learners and maze controls with stubby ($\beta = 0.1061$, SE = 0.1985, p = 0.598), filopodia ($\beta = -0.4482$, SE = 0.2768, p = 0.12) (Fig. 5b), or double-headed morphologies ($\beta = 0.01005$, SE = 0.02332, p = 0.669) where the average density per 10 μm for double-headed morphologies in response learners was 0.061 ± 0.024 and 0.046 ± 0.02 for maze controls. Likewise, no differences were observed between groups in spine length or spine head width (Fig. 5c and d). Taken together, the results of this study demonstrate overall significant differences in dendritic spine density and spine morphology of medium spiny neurons of response learners, effects which are not specific to brain side or to those cells that express the protein product of the immediate early gene zif 268 after training.

4. Discussion

Our findings suggest that intensive, short-term training on a response learning maze task results in a small but significant increase in dendritic spine density of medium spiny neurons in the dorsolateral striatum of adult rats. These effects were not specific to medium spiny neurons that express the protein product of the immediate early gene zif 268 after training, nor were they specific to a side of the brain (ipsilateral or contralateral to the learned response). Instead, they appear to be general changes induced throughout this population of neurons within the dorsolateral, but not dorsomedial, striatum. In addition to overall increases in dendritic spine density with response learning, there was a more specific increase in the number of spines categorized as mushroom-shaped, along with a decrease in those categorized as thin-shaped, in response learners compared to maze controls.

Our findings on short-term training inducing increased dendritic spine density on medium spiny neurons are in direct contrast to those from a recent report indicating no such changes after response training on a similar maze (Hawes et al., 2015). There are many possible reasons for these differences, among them are the use of a different strain of rats (Long Evans versus Sprague Dawley) and a different method for labeling medium spiny neurons (biocytin versus DiI). Regarding this latter point, it may be relevant that our spine density values were more than double than those of the previous study (Hawes et al., 2015), raising the possibility of differential labeling between methods. It is also possible that differences in the duration of the training paradigm were important for the detection of a spine density difference in our study. Our response trained group received 2 days of response learning training and testing (in addition to a habituation day), with a total of approximately 51 response learning trials, while the previous study examined rats in an early training paradigm with 4–6 days of training, and on average 24 trials overall. Dendritic spine growth on medium spiny neurons may occur only very early on in training and it may require massed trials. In this regard, it may be relevant that our findings are consistent with a study examining inhibitory avoidance training using aversive stimuli, which showed that only pairings of stimuli with intensive shocks were sufficient to induce dendritic spine growth (Bello-Medina, Flores, Quirarte, McGaugh, & Prado Alcalá, 2016). Nonetheless, our findings indicate that under certain circumstances, dorsolateral striatum medium spiny neurons undergo dendritic spine growth after intensive, short-term maze response training.

Our data suggest that the observed increase in overall dendritic spine density of medium spiny neurons in response learners with short-term intensive training reflects a learning-induced increase in both density and percentage of mushroom-shaped spines. Dendritic spine shapes have been studied extensively and appear to represent different stages of spine formation, as well as different functional states (Bosch & Hayashi, 2012; Bourne & Harris, 2007). Filopodia and thin spines are

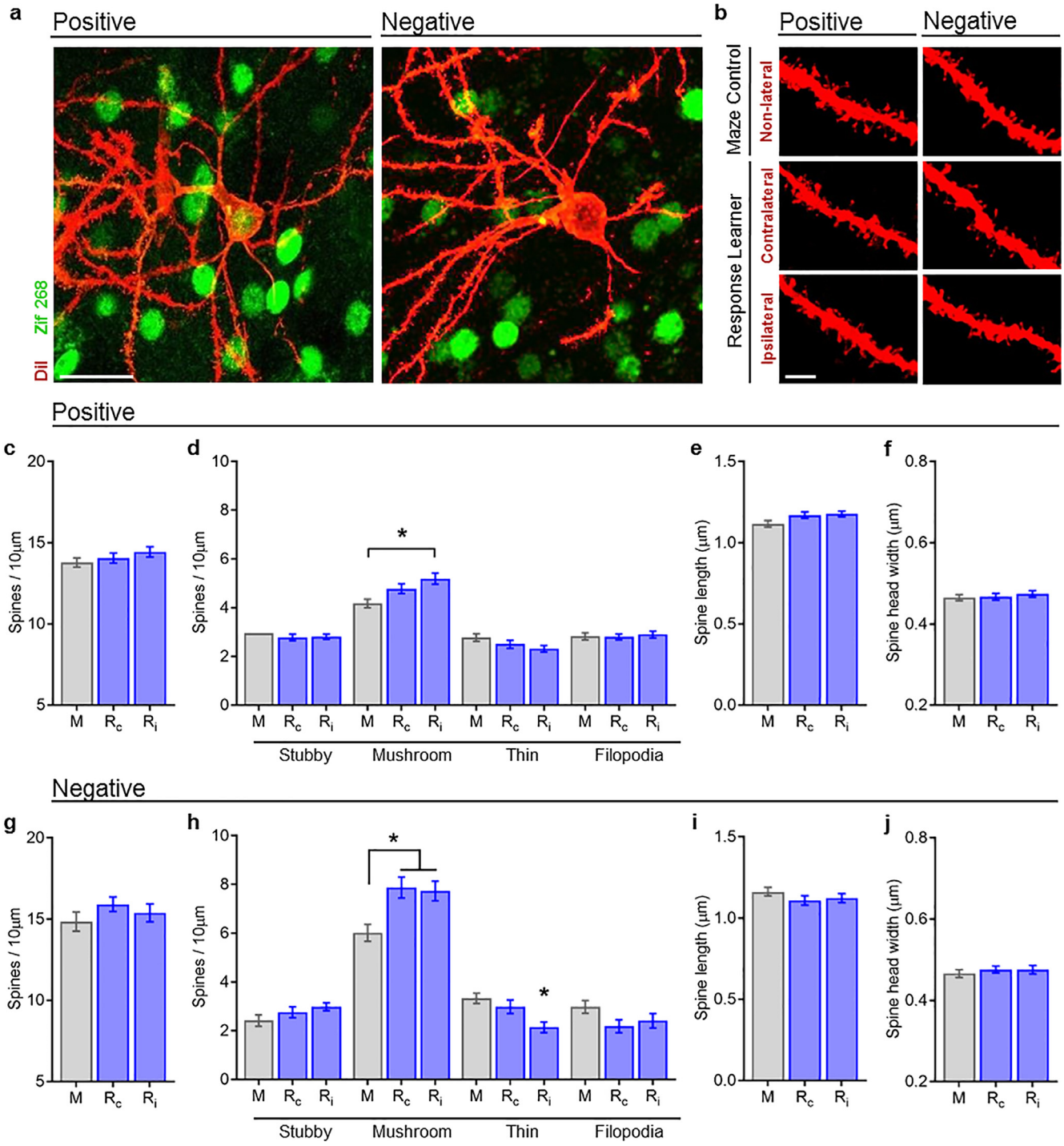

Fig. 4. The dendritic spine density increase after early response learning is not specific to zif 268-positive and zif 268-negative medium spiny neurons. (a) Representative images of DiI-labeled (red) medium spiny neurons, positive (left) or negative (right) for immediate early gene zif 268 (green). Scale bar equals 25 µm. (b) Representative DiI-labeled (red) dendritic segments of medium spiny neurons, positive (left) or negative (right) for zif 268 (not depicted in segment images), from non-lateralized hemisphere of maze control (top row), contralateral hemisphere of response learners (middle row) and ipsilateral hemisphere of response learners (bottom row). Scale bar equals 5 µm. *c-f,* Zif 268-positive spine measurement data for density per 10 µm (c), morphology density per 10 µm (d), length in µm (e), and head width in µm (f). Maze controls (grey) show significantly fewer mushroom spines than do response learners' ipsilateral hemisphere (blue). (g–j) Zif 268-negative spine measurement data for density per 10 µm (g), morphology density per 10 µm (h), length in µm (i), and head width in µm (j). Maze controls show significantly fewer mushroom spines than response learners. Response learner ipsilateral hemisphere shows significantly fewer thin spines than both maze controls and response learner contralateral hemisphere (h) (n = 9–10). $^{*}p < 0.05$; M, maze control; R_c, response learner contralateral; R_i, response learner ipsilateral. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

believed to be transient extensions that are sometimes, but not always, associated with synapses. These spine types are often described as indicators of plasticity, representing dendritic outgrowths that have the potential to develop into more mature spines with stable synapses in response to certain cues (Segal, 2001; Yoshihara, De Roo, & Muller, 2009). Mushroom, stubby, and double-headed spines, the last of which occur at very low frequency, are thought to represent more mature postsynaptic sites, with well-developed stable synapses (Bourne &

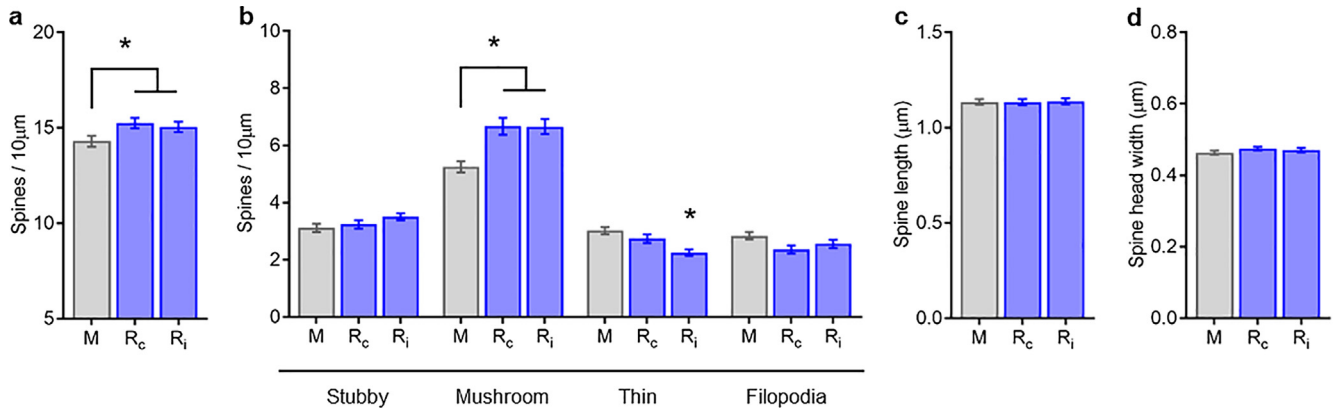

Fig. 5. The zif 268-positive and zif 268-negative medium spiny neuron population combined replicates the dendritic spine density increase seen in early training. (a) Overall spine density difference seen between maze controls and response learners in zif 268-positive and zif 268-negative combined spine measurement data for density per 10 µm. (b) Stubby, mushroom, thin, and filopodia spine morphology density per 10 µm. Maze controls show significantly fewer mushroom spines than response learners. Response learner ipsilateral hemisphere shows significantly fewer thin spines than both maze controls and response learner contralateral hemisphere (n = 9–10). (c) Dendritic spine length in µm, and (d) spine head width in µm. Bar graph error bars represent ± SEM. $^{*}p < 0.05$; M, maze control; R_c, response learner contralateral; R_i, response learner ipsilateral.

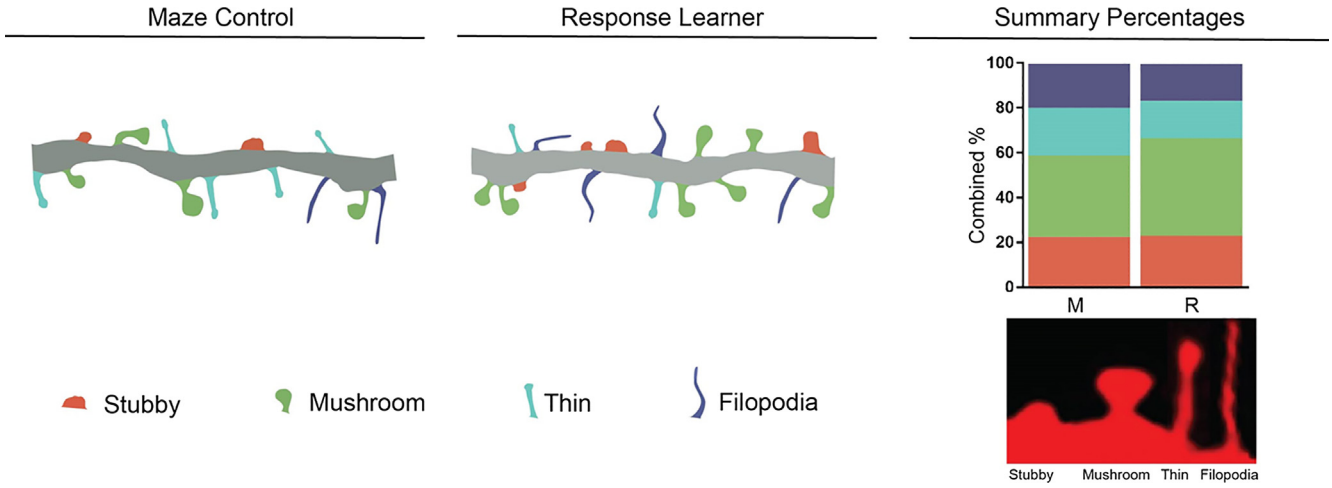

Fig. 6. Summary of response learning effects on number and morphology of medium spiny neuron dendritic spines. Early trained response learners show increased overall spine density, with more mushroom spines, compared to maze controls. Combined percentages of spine morphologies are displayed in bar graphs on the right. Representative stubby, mushroom, thin, and filopodia spine types are shown in the confocal image on the bottom right. M, maze control; R, response learner.

Harris, 2007). The increase in mushroom spine density we observed in the response learners most likely represents a transition of immature spines to mature spines. Given our observation of a concomitant decrease in thin spine density, this appears to be a plausible scenario. However, given the overall small but significant increase in overall spine density, independent of spine morphology subtypes, it is likely that response learning also produced some spines de novo.

Previous studies indicate that the dorsolateral striatum is required for response learning whereas the dorsomedial striatum is required for place learning (Packard & Knowlton, 2002; Yin & Knowlton, 2006). At the outset of maze navigation training, it has been suggested that although the dorsomedial striatum is not critical for the acquisition of response learning (Packard & McGaugh, 1996), habits are formed through transitions from goal-directed behavior to routine responses (Furlong, Jayaweera, Balleine, & Corbit, 2014; Yin, Knowlton, & Balleine, 2004, 2006). Given the engagement of the dorsomedial striatum in early response learning maze training, we examined this brain region as well as the dorsolateral striatum. We did not observe any change in dendritic spine density in response learners compared to maze controls for dorsomedial medium spiny neurons. This lack of an effect in the dorsomedial striatum may seem unexpected given the short training paradigm, however the task we used forced rats to use a non-spatial strategy from the outset, turning direction was predetermined before the training period, and there were no extra-maze cues. Thus, the stimulation of dendritic spine growth may require intensive engagement of neurons in a way that is critical for acquisition of the response.

A previous study examining the effects of enriched environment living compared to caged controls on medium spiny neuron dendritic spine density in the dorsolateral striatum did not reveal overall differences in spine density but observed an increase in double-headed spines (Comery, Stamoudis, Irwin, & Greenough, 1996), an effect we did not observe, likely due to the fact that our controls were also enriched, i.e., they were maze-exposed. Given that our controls were exposed to the maze in a time- and reinforcement-yoked manner, it is unlikely that the changes in dendritic spines we observed in the response learning group occurred as a result of general enrichment, as opposed to specific maze training. However, the lack of an increase in dendritic spine density specific to zif 268-positive cells or to brain side was unexpected and suggests generalized growth response to the learning task. Although studies have shown that activated neurons express zif 268 mRNA and protein (Havik, Røkke, Bårdsen, Davanger, & Bramham, 2003; Murphy et al., 1991) and that this transcription factor is associated with learning and memory (Hall, Thomas, & Everitt, 2001), it is possible that other immediate early genes may better label medium spiny neurons

that are more specifically engaged in the response learning task. It is also possible that the timing of perfusion relative to the activation of the relevant neuron types did not capture the neuronal subpopulation most engaged in the acquisition of the task. While we selected the one hour post-testing time point to examine the zif 268-positive neuronal population at a time shortly after the rats had acquired the learned response, it remains possible that a different subpopulation of neurons was activated earlier in training which may have accounted for a greater degree of dendritic spine growth. Finally, it remains possible that different subsets of medium spiny neurons, such as those that express different dopamine receptors, might reveal subtype specific dendritic spine effects. Our results, however, do not distinguish between these subtypes and suggest that the effect may be general to the entire population of medium spiny neurons in the dorsolateral striatum.

Some studies examining the effects of other types of learning on dendritic spine density and morphology in different brain regions, including the hippocampus and cerebellum, have revealed growth effects that are transient. That is, training initially stimulates dendritic spine growth and maturation, but these measures regress back to the control state even with continued training (Knafo, Libersat, & Barkai, 2005; O'Malley, O'Connell, Murphy, & Regan, 2000). The majority of studies, however, have demonstrated that increases in spine growth and maturation with learning persist over time (González-Tapia et al., 2015; Ibias et al., 2015; Kuhlman et al., 2014; Lee, Jung, Arii, Imoto, & Rhyu, 2007; Ma et al., 2016; Moser et al., 1994; Uriarte, Ogundele, & Pardo, 2017). Regardless of the persistence of spine changes over time, several lines of evidence suggest that new dendritic spine growth and associated new synapses are substrates for learning and memory formation in other brain regions, including the hippocampus, neocortex, and cerebellum. First, training and synaptic plasticity have been associated with spine and synapse growth (González-Tapia et al., 2015; Hill & Zito, 2013; Kuhlman et al., 2014; Lee et al., 2007; Ma et al., 2016; Moser et al., 1994; Uriarte et al., 2017). Second, spine density has been shown to correlate positively with performance on spatial navigation and motor tasks (González-Tapia et al., 2015; Mahmmoud et al., 2015). Third, preventing the formation of new spines and synapses can block formation of some of these types of memories (Liston et al., 2013). Our results suggest a link may exist between new dendritic spine growth in the dorsolateral striatum and stereotyped response memories, as well. However, since these results are correlational, the extent to which new dendritic spines are required for response acquisition, habit formation, and the maintenance of these behaviors remains unknown.

Collectively, these results suggest that short-term, intensive training on a response learning paradigm increases the density of dendritic spines on dorsolateral striatum medium spiny neurons, with the most robust changes occurring among those with a mushroom morphology, compared to maze controls. This effect appears to be general in that it is not dependent on zif 268 expression by the neuron or the side of the brain. The extent to which these structural changes underlie altered behavioral capabilities remains to be determined.

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Conflict of interest

None.

References

Bailey, C. H., Kandel, E. R., & Harris, K. M. (2015). Structural components of synaptic plasticity and memory consolidation. *Cold Spring Harbor Perspectives in Biology, 7*, a021758.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). _lme4: Linear mixed-effects models using Eigen and S4_. R package version 1.1-7, http://CRAN.R-project.org/package=lme4.

Bello-Medina, P. C., Flores, G., Quirarte, G. L., McGaugh, J. L., & Prado Alcalá, R. A. (2016). Mushroom spine dynamics in medium spiny neurons of dorsal striatum associated with memory of moderate and intense training. *Proceedings of the National Academy of Sciences of the United States of America, 113*, E6516–E6525.

Bosch, M., & Hayashi, Y. (2012). Structural plasticity of dendritic spines. *Current Opinion in Neurobiology, 22*, 383–388.

Bourne, J., & Harris, K. M. (2007). Do thin spines learn to be mushroom spines that remember? *Current Opinion in Neurobiology, 17*, 381–386.

Chang, Q., & Gold, P. E. (2003). Switching memory systems during learning: Changes in patterns of brain acetylcholine release in the hippocampus and striatum in rats. *Journal of Neuroscience, 23*, 3001–3005.

Chang, H. T., Wilson, C. J., & Kitai, S. T. (1982). A Golgi study of rat neostriatal neurons: Light microscopic analysis. *The Journal of Comparative Neurology, 208*, 107–126.

Comery, T. A., Stamoudis, C. X., Irwin, S. A., & Greenough, W. T. (1996). Increased density of multiple-head dendritic spines on medium-spiny neurons of the striatum in rats reared in a complex environment. *Neurobiology of Learning and Memory, 66*, 93–96.

Cui, G., Jun, S. B., Jin, X., Pham, M. D., Vogel, S. S., Lovinger, D. M., & Costa, R. M. (2013). Concurrent activation of striatal direct and indirect pathways during action initiation. *Nature, 494*, 238–242.

Devan, B. D., McDonald, R. J., & White, N. M. (1999). Effects of medial and lateral caudate-putamen lesions on place- and cue-guided behaviors in the water maze: Relation to thigmotaxis. *Behavioural Brain Research, 100*, 5–14.

Feldman, D. E. (2009). Synaptic mechanisms for plasticity in neocortex. *Annual Review of Neuroscience, 32*, 33–55.

Fu, M., Yu, X., Lu, J., & Zuo, Y. (2012). Repetitive motor learning induces coordinated formation of clustered dendritic spines in vivo. *Nature, 483*, 92–95.

Furlong, T. M., Jayaweera, H. K., Balleine, B. W., & Corbit, L. H. (2014). Binge-like consumption of a palatable food accelerates habitual control of behavior and is dependent on activation of the dorsolateral striatum. *Journal of Neuroscience, 34*, 5012–5022.

Gill, K. M., Bernstein, I. L., & Mizumori, S. J. (2007). Immediate early gene activation in hippocampus and dorsal striatum: Effects of explicit place and response learning. *Neurobiology of Learning and Memory, 87*, 583–596.

Gipson, C. D., & Olive, M. F. (2017). Structural and functional plasticity of dendritic spines - root or result of behavior? *Genes, Brain and Behavior, 16*, 101–117.

González-Tapia, D., Velázquez-Zamora, D. A., Olvera-Cortés, M. E., & González-Burgos, I. (2015). The motor learning induces plastic changes in dendritic spines of Purkinje cells from the neocerebellar cortex of the rat. *Restorative Neurology and Neuroscience, 33*, 639–645.

Hall, J., Thomas, K. L., & Everitt, B. J. (2001). Cellular imaging of zif268 expression in the hippocampus and amygdala during contextual and cued fear memory retrieval: Selective activation of hippocampal CA1 neurons during the recall of contextual memories. *Journal of Neuroscience, 21*, 2186–2193.

Håvik, B., Røkke, H., Bårdsen, K., Davanger, S., & Bramham, C. R. (2003). Bursts of high-frequency stimulation trigger rapid delivery of pre-existing alpha-CaMKII mRNA to synapses: A mechanism in dendritic protein synthesis during long-term potentiation in adult awake rats. *European Journal of Neuroscience, 17*, 2679–2689.

Hawes, S. L., Evans, R. C., Unruh, B. A., Benkert, E. E., Gillani, F., Dumas, T. C., & Blackwell, K. T. (2015). Multimodal plasticity in dorsal striatum while learning a lateralized navigation task. *Journal of Neuroscience, 35*, 10535–10549.

Hill, T. C., & Zito, K. (2013). LTP-induced long-term stabilization of individual nascent dendritic spines. *Journal of Neuroscience, 33*, 678–686.

Hu, D., & Amsel, A. (1995). A simple test of the vicarious trial-and-error hypothesis of hippocampal function. *Neurobiology, 92*, 5506–5509.

Íbias, J., Soria-Molinillo, E., Kastanauskaite, A., Orgaz, C., DeFelipe, J., Pellón, R., & Miguéns, M. (2015). Schedule-induced polydipsia is associated with increased spine density in dorsolateral striatum neurons. *Neuroscience, 300*, 238–245.

Jasinska, M., Siucinska, E., Jasek, E., Litwin, J. A., Pyza, E., & Kossut, M. (2016). Effect of associative learning on memory spine formation in mouse barrel cortex. *Neural Plasticity, 2016*, 9828517.

Knafo, S., Libersat, F., & Barkai, E. (2005). Dynamics of learning-induced spine redistribution along dendrites of pyramidal neurons in rats. *European Journal of Neuroscience, 21*, 927–935.

Kötter, R. (1994). Postsynaptic integration of glutamatergic and dopaminergic signals in the striatum. *Progress in Neurobiology, 44*, 163–196.

Kravitz, A. V., Freeze, B. S., Parker, P. R., Kay, K., Thwin, M. T., Deisseroth, K., & Kreitzer, A. C. (2010). Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature, 466*, 622–626.

Kuhlman, S. J., O'Connor, D. H., Fox, K., & Svoboda, K. (2014). Structural plasticity within the barrel cortex during initial phases of whisker-dependent learning. *Journal of Neuroscience, 34*, 6078–6083.

Lee, K. J., Jung, J. G., Arii, T., Imoto, K., & Rhyu, I. J. (2007). Morphological changes in dendritic spines of Purkinje cells associated with motor learning. *Neurobiology of Learning and Memory, 88*, 445–450.

Lee, K. W., Kim, Y., Kim, A. M., Helmin, K., Nairn, A. C., & Greengard, P. (2006). Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 3399–3404.

Leuner, B., Falduto, J., & Shors, T. J. (2003). Associative memory formation increases the observation of dendritic spines in the hippocampus. *Journal of Neuroscience, 23*,

659–665.
Lim, S. A., Kang, U. J., & McGehee, D. S. (2014). Striatal cholinergic interneuron regulation and circuit effects. *Frontiers in Synaptic Neuroscience, 6*, 22.
Liston, C., Cichon, J. M., Jeanneteau, F., Jia, Z., Chao, M. V., & Gan, W. B. (2013). Circadian glucocorticoid oscillations promote learning-dependent synapse formation and maintenance. *Nature Neuroscience, 16*, 698–705.
Llinas, R., & Sotelo, C. (1992). *The cerebellum revisited.* NY: Springer-Verlag.
Ma, L., Qiao, Q., Tsai, J. W., Yang, G., Li, W., & Gan, W. B. (2016). Experience-dependent plasticity of dendritic spines of layer 2/3 pyramidal neurons in the mouse cortex. *Developmental Neurobiology, 76*, 277–286.
Mahmmoud, R. R., Sase, S., Aher, Y. D., Sase, A., Gröger, M., Mokhtar, M., ... Lubec, G. (2015). Spatial and working memory is linked to spine density and mushroom spines. *PLoS One, 10*, e0139739.
Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., & Wu, C. (2004). Interneurons of the neocortical inhibitory system. *Nature Reviews Neuroscience, 5*, 793–807.
Moser, M. B., Trommald, M., & Andersen, P. (1994). An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 12673–12675.
Muenzinger, K. F. (1938). Vicarious trial and error at a point of choice. I. A general survey of its relation to learning efficiency. *Journal of Genetic Psychology, 53*, 75–86.
Muenzinger, K. F., & Gentry, E. (1931). Tone discrimination in white rats. *Journal of Comparative Psychology, 12*, 195–206.
Murakoshi, H., & Yasuda, R. (2012). Postsynaptic signaling during plasticity of dendritic spines. *Trends in Neurosciences, 35*, 135–143.
Murphy, T. H., Worley, P. F., Nakabeppu, Y., Christy, B., Gastel, J., & Baraban, J. M. (1991). Synaptic regulation of immediate early gene expression in primary cultures of cortical neurons. *Journal of Neurochemistry, 57*, 1862–1872.
Nishiyama, N., Colonna, J., Shen, E., Carrillo, J., & Nishiyama, H. (2014). Long-term in vivo time-lapse imaging of synapse development and plasticity in the cerebellum. *Journal of Neurophysiology, 111*, 208–216.
O'Malley, A., O'Connell, C., Murphy, K. J., & Regan, C. M. (2000). Transient spine density increases in the mid-molecular layer of hippocampal dentate gyrus accompany consolidation of a spatial learning task in the rodent. *Neuroscience, 99*, 229–232.
Packard, M. G., & Knowlton, B. J. (2002). Learning and memory functions of the basal ganglia. *Annual Review of Neuroscience, 25*, 563–593.
Packard, M. G., & McGaugh, J. L. (1996). Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiology of Learning and Memory, 65*, 65–72.
Park, M., Salgado, J. M., Ostroff, L., Helton, T. D., Robinson, C. G., Harris, K. M., & Ehlers, M. D. (2006). Plasticity-induced growth of dendritic spines by exocytic trafficking from recycling endosomes. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 8456–8460.
Pelkey, K. A., Chittajallu, R., Craig, M. T., Tricoire, L., Wester, J. C., & McBain, C. J. (2017). Hippocampal GABAergic inhibitory interneurons. *Physiological Reviews, 97*, 1619–1747.
Peters, A., & Kaiserman-Abramof, I. R. (1970). The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites and spines. *The American Journal of Anatomy, 127*, 321–355.
Redish, A. D. (2016). Vicarious trial and error. *Nature Reviews Neuroscience, 17*, 147–159.
Restivo, L., Vetere, G., Bontempi, B., & Ammassari-Teule, M. (2009). The formation of recent and remote memory is associated with time-dependent formation of dendritic spines in the hippocampus and anterior cingulate cortex. *Journal of Neuroscience, 29*, 8206–8214.
Rogue, P., & Vincendon, G. (1992). Dopamine D2 receptor antagonists induce immediate early genes in the rat striatum. *Brain Research Bulletin, 29*, 469–472.
RStudio Team (2016). RStudio: Integrated Development for R. Boston, MA: RStudio, Inc. URL http://www.rstudio.com/.
Sanders, J., Cowansage, K., Baumgärtel, K., & Mayford, M. (2012). Elimination of dendritic spines with long-term memory is specific to active circuits. *Journal of Neuroscience, 32*, 12570–12578.
Segal, M. (2001). Rapid plasticity of dendritic spine: Hints to possible functions? *Progress in Neurobiology, 63*, 61–70.
Swanson, A. M., DePoy, L. M., & Gourley, S. L. (2017). Inhibiting Rho kinase promotes goal-directed decision making and blocks habitual responding for cocaine. *Nature Communications, 8*, 1861.
Tolman, E. C. (1938). The determiners of behavior at a choice point. *Psychological Review, 45*, 1–41.
Tolman, E. C. (1939). Prediction of vicarious trial and error by means of the schematic sowbug. *Psychological Review, 46*, 318–336.
Uriarte, M., Ogundele, O. M., & Pardo, J. (2017). Long-lasting training in the Barnes maze prompts hippocampal spinogenesis and habituation in rats. *Neuroreport, 28*, 307–312.
van der Meer, M. A. A., & Redish, A. D. (2010). Triple dissociation of information processing in decision-making systems. *Neuroscientist, 18*, 342–359.
Vetere, G., Restivo, L., Cole, C. J., Ross, P. J., Ammassari-Teule, M., Josselyn, S. A., & Frankland, P. W. (2003). Spine growth in the anterior cingulate cortex is necessary for the consolidation of contextual fear memory. *Journal of Neuroscience, 23*, 659–665.
Yin, H. H., & Knowlton, B. J. (2006). The role of the basal ganglia in habit formation. *Nature Reviews Neuroscience, 7*, 464–476.
Yin, H. H., Knowlton, B. J., & Balleine, B. W. (2004). Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. *European Journal of Neuroscience, 19*, 181–189.
Yin, H. H., Knowlton, B. J., & Balleine, B. W. (2006). Inactivation of dorsolateral striatum enhances sensitivity to changes in the action-outcome contingency in instrumental conditioning. *Behavioural Brain Research, 166*, 189–196.
Yoshihara, Y., De Roo, M., & Muller, D. (2009). Dendritic spine formation and stabilization. *Current Opinion in Neurobiology, 19*, 146–153.
Yuste, R. (2011). Dendritic spines and distributed circuits. *Neuron, 71*, 772–781.
Yuste, R., & Bonhoeffer, T. (2001). Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annual Review of Neuroscience, 24*, 1071–1089.
Yuste, R., & Bonhoeffer, T. (2004). Genesis of dendritic spines: Insights from ultrastructural and imaging studies. *Nature Reviews Neuroscience, 5*, 24–34.