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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