Behavioural Brain Research 299 (2016) 122–131

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

How does environmental enrichment reduce repetitive motor behaviors? Neuronal activation and dendritic morphology in the indirect basal ganglia pathway of a mouse model

Allison R. Bechard[a,*], Nadia Cacodcar[a], Michael A. King[b], Mark H. Lewis[a,c]

[a] Department of Psychology, University of Florida, Gainesville, FL, USA
[b] Department of Pharmacology, University of Florida, Gainesville, FL, USA
[c] Department of Psychiatry, McKnight Brain Institute, University of Florida, Gainesville, FL, USA

HIGHLIGHTS

- EE reduced repetitive motor behaviors in a deer mouse model.
- EE-induced attenuation of repetitive behavior was associated with increased activation of the indirect basal ganglia pathway.
- EE-induced attenuation of repetitive behavior was associated with increased dendritic spine densities in the STN.

ARTICLE INFO

Article history:
Received 18 September 2015
Received in revised form
19 November 2015
Accepted 20 November 2015
Available online 24 November 2015

Keywords:
Animal models
Basal ganglia circuitry
Subthalamic nucleus
Cytochrome oxidase
Golgi-Cox histochemistry
Deer mice

ABSTRACT

Repetitive motor behaviors are observed in many neurodevelopmental and neurological disorders (e.g., autism spectrum disorders, Tourette syndrome, fronto-temporal dementia). Despite their clinical importance, the neurobiology underlying these highly stereotyped, apparently functionless behaviors is poorly understood. Identification of mechanisms that mediate the development of repetitive behaviors will aid in the discovery of new therapeutic targets and treatment development. Using a deer mouse model, we have shown that decreased indirect basal ganglia pathway activity is associated with high levels of repetitive behavior. Environmental enrichment (EE) markedly attenuates the development of such aberrant behaviors in mice, although mechanisms driving this effect are unknown. We hypothesized that EE would reduce repetitive motor behaviors by increasing indirect basal ganglia pathway function. We assessed neuronal activation and dendritic spine density in basal ganglia of adult deer mice reared in EE and standard housing. Significant increases in neuronal activation and dendritic spine densities were observed only in the subthalamic nucleus (STN) and globus pallidus (GP), and only for those mice that exhibited an EE-induced decrease in repetitive motor behavior. As the STN and GP lie within the indirect pathway, these data suggest that EE-induced attenuation of repetitive motor behaviors is associated with increased functional activation of the indirect basal ganglia pathway. These results are consistent with our other findings highlighting the importance of the indirect pathway in mediating repetitive motor behaviors.

1. Introduction

Repetitive motor behaviors are rigid patterns of behavior that serve no apparent function [42]. These problem behaviors manifest in many clinical populations, notably neurodevelopmental disorders such as autism spectrum disorders and intellectual and developmental disability [6]. Several neurological and psychiatric disorders including fronto-temporal dementia, obsessive compulsive disorder (OCD), schizophrenia, Tourette's syndrome, Parkinson's and Huntington's diseases have repetitive motor behaviors as part of their clinical presentation as well [59,35]. Repetitive motor behaviors also develop as a consequence of early environmental deprivation, including congenital blindness and impoverished environments [11,61]. In spite of the large number of affected people, the neurobiological or pathophysiological basis of these behaviors is not well understood. In neurodevelopmental disorders, evidence is limited to a small number of MRI studies that have demonstrated volumetric differences in basal ganglia, mostly caudate/putamen, related to repetitive behavior

* Corresponding author at: 945 Center Dr, P.O. Box 112250, Gainesville, FL, 32611-2250, USA. Fax: +13522940425.
E-mail address: abechard@ufl.edu (A.R. Bechard).

http://dx.doi.org/10.1016/j.bbr.2015.11.029
0166-4328/

[64,22,60,36]. As a consequence of very limited information on the underlying neurobiology, effective pharmacological treatments are largely lacking, particularly in neurodevelopmental disorders. Identifying the underlying mechanisms for the development of repetitive motor behaviors will promote identification of new therapeutic targets and treatment development.

Animal models of repetitive behavior provide a valuable approach to identify underlying mechanisms of repetitive behavior in response to varying environments. Repetitive motor behaviors can be induced in animals in a variety of ways including by pharmacological agents (e.g., amphetamine), CNS insult (e.g., deletion of genes coding for Shank3, MECP-2, SAP-AP3) and environmental restriction (e.g., standard laboratory caging) [41,43,47,3]. Deer mice (*Peromyscus maniculatus*) have proven to be a useful model of repetitive behavior induced by environmental restriction (see review by Lewis et al. [43]). As a consequence of standard laboratory caging, deer mice exhibit high levels of repetitive hindlimb jumping and backward somersaulting, apparent by the time of weaning and persisting across adulthood [50,57]. Furthermore, deer mice reared in enriched environments show significant attenuation of the development of repetitive motor behaviors [56,73,17].

Environmental enrichment (EE) in rodents includes increased social and spatial density as well as exposure to novelty and complexity, usually in the form of toys, tunnels, and nesting material. Increased opportunity for exercise (e.g., a running wheel) is also a mainstay of EE. Well-studied in the context of learning and memory, EE increases neurogenesis and synaptogenesis [75] as well as cortical thickness, dendritic spine density and length, synaptic plasticity, and resistance to disease [34,52]. A range of behavioral domains are also affected by EE including cognitive, social, and emotional functioning as well as aberrant behaviors [49,7]. Attenuation of repetitive behavior is a robust behavioral effect of EE, seen not only in deer mice, but across most captive species [41,47]. To date, however, the mechanisms underlying this effect are largely unknown. Previous work in deer mice suggested that EE-induced attenuation of repetitive motor behaviors was associated with increased neuronal activation and dendritic spine density in basal ganglia [73,72]. Importantly, EE effects on neurobiological outcomes were found only for those mice exhibiting EE-induced attenuation of repetitive behavior.

Although this earlier work implicated the basal ganglia, it did not address selective alterations of specific basal ganglia pathways. The basal ganglia include the striatum, globus pallidus (GP), subthalamic nucleus (STN) and substantia nigra (SN). The striatum is the main input structure of the basal ganglia and receives projections from the sensory-motor and associative cortical areas, and projects to nuclei via the direct or indirect pathway of the basal ganglia. These projections converge on the output nucleus, the SN, relay to the thalamus and then back to the cortex to complete the loop. The monosynaptic direct pathway projects from striatum to SN pars reticulata (SNR), whereas the indirect pathway projects from striatum to GP which in turn projects to STN before converging on SNR [63]. Appropriate selection, activation and suppression of movement are dependent on the coordination of the direct and indirect basal ganglia pathways, which classically were thought to function in an antagonistic fashion [1]. More recent evidence reveals a dynamic interplay between the basal ganglia pathways with concomitant activity during action sequence initiation but differential encoding of action sequences [27], and increased complexity of neuronal cell populations within a given region [76,2,45]. Direct pathway neurons facilitate selection of relevant motor programs whereas the indirect pathway functions to suppress competing motor programs. In addition, a direct connection between the cortex and STN, known as the hyperdirect pathway, although largely understudied, is thought to modulate response inhibition in situations of conflict [26,25]. An imbalance in the direct and indirect basal ganglia pathways has been implicated in the dysregulation of cortico-striato-thalamo-cortical circuitry associated with both hyperkinetic and hypokinetic movement disorders [13].

In the deer mouse model of repetitive behavior, our work has suggested a functional imbalance of the direct and indirect basal ganglia pathways due to a hypoactivation of the indirect pathway. For example, we found a significant reduction in striatal enkephalin, a marker of indirect basal ganglia pathway neurons, in high versus low repetitive behavior mice with no difference in striatal dynorphin, a marker of direct pathway neurons [58]. In addition, a significant inverse correlation was found between repetitive behavior scores and striatal enkephalin content [58]. We also showed that neuronal activity in the STN was reduced in mice with high versus low levels of repetitive motor behavior [67–69]. Further, a brief period of EE that was effective in reducing repetitive behavior development was associated with increased neuronal activation in the STN [68,69]. Targeting striatal indirect pathway neurons with pharmacological agents designed to increase indirect pathway activation substantially reduced repetitive motor behavior in deer mice [68,69].

In the present study, we conducted two experiments to assess the function of the indirect pathway in the EE-induced attenuation of the development of repetitive motor behaviors. We hypothesized that such attenuation is associated with increased neuronal activation of the indirect basal ganglia pathway, an outcome mediated by increased dendritic spine density. In Study 1, we compared neuronal metabolic activation of basal ganglia nuclei in adult deer mice reared in EE to mice reared in standard housing. Neuronal activation is tightly coupled with oxidative energy metabolism, and can be indexed using cytochrome oxidase (CO) histochemistry [12]. We measured CO as an indicator of long-term neuronal metabolic activity in the dorsal lateral striatum (DLS), GP, STN, SNR, SNC, motor cortex and CA1 region of the hippocampus (HPC). In Study 2, we compared dendritic morphology in basal ganglia nuclei of adult deer mice reared in EE or standard laboratory cages. The dendritic surface receives over 95% of the synapses on a neuron, aided by specialized protrusions (i.e., spines) that act as the basic functional unit of integration for neuronal circuits. Dendritic spines mediate fast excitatory transmission, are heterogeneous in morphology, and modifiable by experience and activity [46]. Experience-dependent dendritic plasticity is a sensitive index for inferring synapse number and strength, and dendritic remodeling can change the functional properties of a neuron [18,34]. A number of earlier studies investigating morphological plasticity have found increased dendritic spine density following EE exposure in a variety of brain regions important in the processing of environmental stimuli (e.g., [8,9,29,30] reviewed by Markham and Greenough [46]). More recently, dendritic remodeling as a consequence of EE has been related to tasks of learning and memory [10] and pathology of Huntington's and Parkinson's diseases [66,51,32]. To our knowledge, however, only our own work has examined dendritic morphological differences in the basal ganglia as a function of EE-induced attenuation of repetitive motor behaviors [72].

2. Materials and methods

2.1. Animals

All procedures were performed in accordance with NIH Guidelines for the Care and Use of Laboratory Animals and approved by the University of Florida Institutional Animal Care and Use Committee. Deer mice were bred and housed in our colony room at the University of Florida, maintained at 70–75 °F and 50–70% humidity, under a 16:8 light:dark cycle, with lights off at 10:00 am. All home environments had access to rodent chow (Teklad) and water *ad lib*, and two Nestlet squares for nest construction. Offspring of