Effects of Touchscreen Training on Hippocampal Plasticity with BDNF as a Mediator

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Abstract: Hippocampus is the hub in neural system and the focus in cognitive science as it is essential for learning and memory. Neurogenesis opened a new chapter on hippocampus plasticity and abundant researches have suggested that neurogenesis could be regulated by various in vivo and in vitro factors such as training. In molecular level, brain-derived neurotropic factor (BDNF) promote neuroplasticity and contributes to learning and memory. In the present study, the touchscreen-equipped system was introduced and the mechanisms regulating learning and memory and the dynamic interplay between touchscreen training, hippocampal neurogenesis, learning and memory and the role of BDNF signaling were explored. Additionally, we discussed how to promote learning and memory through optimizing the conditions of visual stimuli and manipulating the plasticity of hippocampus by BDNF signaling. This could provide new perspectives in effective therapeutic strategies for cognitive dysfunction resulting from neurodegenerative, neuropsychiatric and neurodevelopment disorders.
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Introduction

Neurogenesis, the regeneration and maturation of new neurons, is highly plastic. The hippocampus-dependent learning, such as pattern separation, environmental enrichment could promote hippocampal neurogenesis and facilitate learning and memory. Molecularly, the up-regulation of brain-derived neurotrophic factor (BDNF)-meditated signal transduction pathways could improve learning and memory.[1] In the present review, we will introduce the touchscreen equipped visual recognition system, and explore the effect of touchscreen training on hippocampal plasticity, learning and memory, as well as the role of BDNF signaling. Additionally, we will discuss how to promote learning and memory through regulating the hippocampus plasticity and BDNF signaling as well as molecular and cellular mechanism. This is of great significance for treatment of neurological and psychiatric disorders.

The touchscreen-equipped training and learning system

Currently, the majority of the methods evaluating learning and memory behavior are designed according to the characteristics of animal behavior such as such as foraging, seeking for survival, escaping from harm and preference for bright over darkness. In general, there are two categories of learning, defensive avoidance learning such as shuttling box, climbing pole, electric shock and spatial differentiation memory including a variety of mazes such as the Morris maze, which is regarded as the gold standard to evaluate the behavior of learning and memory. However, it is energy- consuming, time-consuming and is unable to precisely reflect learning scenarios and is unsuitable for older animals.[2] In addition, rapid advances in neuroscience and standards of behavioral analysis have brought new challenges. Therefore, it is both promising and perhaps an impediment for further research to integrate the traditional methods of behavioral research with the current theories and technologies in order to establish an optimized model for in-depth research on the regulation of learning and memory. Only a convenient and practical model based on the training of animals according to their specific lifestyles and habits can meet the needs of the further research on cognition.

Sensory stimulation plays an important role in the survival and development of the individual.[3] Of all the sensory stimuli, visual stimuli are the major source of message received from the outside world, accounting for 80% of all the sensory information inputs.[4] Depending on the visual system, mammals can recognize the features of objects they see, and after filtering, screening, handling and processing of the information by the cerebral cortex, eventually forms a comprehensive visual perception which is of

great importance for survival and development. [4-6] On the other hand, visual processing disorders may eventually lead to learning dysfunction. [7] Visual stimulation has long been one of the most common environmental factors employed in the study of neural networks. Recently, the touchscreen-equipped visual recognition system, a novel visual learning method for rodents, has been increasingly explored and applied. In this model, animals receive visual stimulations of different patterns from the computer screen, and then respond with nose contacting or gently pressing on the switch, combined with reward or punishment, during which food is rewarded immediately when a right distinction is made and punished when a wrong decision is made. It is a self-controlled method integrated with stimulus, responses and feedbacks. The advantages of this system include the fact that it is similar to the method presently used in detecting neuropsychiatric disorders and can be used in both rodents and humans for cognitive testing. This will facilitate the translation between human and animal testing. Visual stimuli are the only stimuli used and stimulus control is significantly greater. Various cognitive tasks can be administered in the same apparatus with similar stimuli responses, and rewards, whereby comparison between tasks are facilitated. It is computer-automated, thus, improving the consistency and accuracy of the task parameters such as stimuli, responses, inter-trial intervals and delays. Moreover, the experimental data are of high precision and can be saved automatically into a linked database for further analysis. Additionally, it minimizes the contact between researchers and animals during the experiment, and is thus, less labor-intensive. It promotes standardization of experiments and data that has been inputted from different laboratories thus can be used for an effective comparison. For example, food-reward is employed instead of negative stimulation such as electric shock and cool water, it is appetitive, and recommended for special animals such as those that are genetically modified. It is of low motoric demand and ease of administration and can be widely applied[8].

Taken together, the touchscreen-equipped visual recognition system is a real-time, simple and effective model to evaluate the cognition of rats under an active and pleasant learning environment. It has been effectively applied in a number of etiological, behavioral, and pharmacological studies for Alzheimer's disease, Huntington's disease, frontotemporal dementia, schizophrenia, aging, stress and drug abuse.[9] Romberg *et.al.* found that early change in attention and executive control of Alzheimer's disease mouse TgCRND8 could be detected by this apparatus, suggesting that it facilitates more professional, comprehensive and complete phenotypic analysis promoting the clinical application of the pre-clinical animal experiments. [10] As noted, this new model provides a relatively high-throughput method for cognitive testing. Therefore, recognizing and optimizing the parameters of the test would enhance the results. It has been reported that there are various methods to optimize the test such as identifying and minimizing spontaneous stimulus bias, optimizing stimulus size, inter-trial interval, trials per session, trial initiation requirements and the selection of appropriate food-rewards.[11] All these parameters are optimized according to specific experiments so as to reach superior tolerability and persistence. What's more, Oomen and Kim *et. al.* reported that trial-unique, delayed nonmatching-to-location touchscreen is sensitivity to dorsal hippocampal dysfunction[12], and continuous trial-unique non-matching-to-location task is sufficiently sensitive to differentiate between dentate gyrus (DG) and CA3 hippocampal subregion contributions to performance[13].

Researchers have suggested that visual learning facilitates the formation of long-term memory through NO-cGMP/cAMP signaling.[14] Both NR2A receptor knockout mice and GLAST knockout mice have impaired visual discrimination. However, neither GluA1 nor M1 receptor knockout mice have any such impairment,[15] the mechanisms underlying visual learning and memory have yet to be fully elucidated.

Hippocampal plasticity and learning and memory

Previous studies indicated that synaptic plasticity is the cellular basis of learning and memory, and long-term potentiation (LTP), which refers to strengthening the function of synapses, could facilitate postsynaptic response, learning and memory.[16] Furthermore, it has been demonstrated that there are a number of neural stem cells (NSCs) in two specific regions of the adult mammalian brain, the subgranular zone of the dentate gyrus and the subventricular zone /olfactory bulb. These observations suggest that the long-held concept that adult neural cells cannot regenerate. In addition, it also suggests that, in addition to synaptic plasticity, the neurogenesis of adult NSCs plays an important role in learning and memory.[17]

As suggested, complicated learning and memory involves coordination of many regions of the brain, with the hippocampus as the hub and that that all the afferent information received by sensory organs must be integrated and stored in the hippocampus with final connections to other regions of the brain for processing. Therefore, hippocampal neurogenesis, the plasticity of adult NSCs and the brain, deserves more attention since it adds new functional neurons to the neural network which is of great importance for learning and memory.

The process of adult hippocampal neurogenesis Adult hippocampal neurogenesis occurs throughout life. There are five developmental stages during adult hippocampal neurogenesis. These include the activation of progenitors (quiescent radial glia-like cells) in the subgranular zone, proliferation of non-radial precursor and intermediate progenitors, generation of neuroblasts, integration of immature neurons and maturation of adult-born dentate granule cells in dentate gyrus (DG).[17] During neurogenesis, new cells undergo a series of morphological and functional changes. [18, 19] Although most of the new cells are removed by apoptosis, 10% to 50% cells survive and become functionally integrated into the already existing networks of the DG, where they may persist for months or even lifetime.[20]

It has been recognized that mature granular cells are typical of the three-stage synaptic hippocampal circuits. Firstly, information from virtually all the higher sensory cortical areas converges on the entorhinal cortex II and III and throughout the perforant pathway conveying to the DG (the first relay station). Information subsequently passes to CA3 (the second relay station), then relays to CA1 (the third station) and transmits back to the entorhinal cortex V.[21] Compared with their mature counterparts, new born neurons have their unique neural circuits. Initially, new neurons receive input information from septal-hippocampal cells, including transient innervations from mature granule cells as well as direct feedback from CA3 area pyramidal neurons. After approximately one month, perirhinal and lateral entorhinal cortex areas of the brain, important to the integration of novel sensory and environmental information, sends substantial input to new granule cells. Therefore, the unique neural circuits results in the special function of new born neurons.[22]

In summary, adult neurogenesis appears to provide a new form of brain plasticity. The supplement of new neurons throughout the lifetime enhance hippocampal information processing efficiently and enhances adult neurogenesis, thus, increasing the pool of responding synapses, strengthen the neural conduction and facilitate long-term memory.

The regulation of hippocampal neurogenesis Animal experiments have demonstrated that normal hippocampal neurogenesis occurs at a low rate in vivo. However, the number of the new-born cells could be changed significantly under certain conditions. It has been demonstrated that hippocampus-dependent learning promoted hippocampal neurogenesis.[23] Other factors such as the age of the immature neurons may also be involved. As previously reported, hippocampus-dependent learning enhanced the survival of the new-born neurons at a certain stage of maturation, 4-week-old new born neurons in mice and 11 to 15-day-old in rats. [6, 24] Thereafter, during the specific period noted above, the activation of the new-born neurons could be detected through the detection of the expression of the early gene c-Fos, arc zif268 and Pcreb133.[25] The activation of new-born neurons could facilitate the consolidation and retrieval of memory. However, before the time window, the neural networks necessary for LTP and the consolidation of memory had not been established. It has been demonstrated that before the time window, neural pathway of the new-born neurons had not been formed, and afterwards the survival of cells was substantially stable that it could not be changed.[26] Therefore, the maturation process of the new-born neurons was a critical factor in hippocampal-dependent learning which could promote survival of the new-born neurons.

Hippocampal neurogenesis could be affected by various internal factors in animal models such as species,[27]strain,[28] gender,[29] age,[30] hormones,[31] pregnancy and lactation. [32] On the other hand, external factors such as brain injury could induce neurogenesis, a self-healing response. However, pressure, intense stress alcoholic binges, depression, epilepsy, cerebral ischemia, Alzheimer's disease, Parkinson's disease and CHARGE syndrome with CD7 mutation inhibit neurogenesis. [33] Neurogenesis has also been demonstrated to be enhanced by administration of drugs including antidepressants,[26] caffeine, modafinil[34], vascular endothelial growth factor[35] and synthetic small molecule Isx-9,[36] etc. Similarly, deleting the pro-apoptotic gene Bax[37] or knocking-in gene MEK5[38] into mice significantly stimulated neurogenesis. Learning with tasks of proper type and complexity,[39] participating in voluntary physical activities[40] and exposing to a particularly rich environment[41, 42] could promote cognition under physiological conditions and even remedy the cognitive dysfunction during pathologic conditions.

At the molecular level, various cytokines, neurotransmitters and their receptors, transcription factors, and the cell-cycle signally pathways (Notch, Shh, Wnts, and BMP) participate in the regulation of neurogenesis.[43] Thus, neurogenesis is regulated by a variety of pathophysiological factors in a complex way.

The effect of hippocampal neurogenesis on learning and memory Investigation of neuronal stems cells (NSCs) provides a new strategy for neural repair as well as antidepressant and senescence delay. It is proposed that the ability of learning and memory could be enhanced through regulating hippocampal neurogenesis[44]. Recent research demonstrated that environmental enrichment, as well as voluntary

physical exercise, could increase adult neurogenesis which accompanies improvement in the ability of learning and memory. Therefore, it has been hypothesized that improvements in learning and memory are the result of increased neurogenesis. Therefore, environmental enrichment or voluntary physical exercise would not lead to improvements in learning and memory when neurogenesis is prevented. Deng *et. al.* reviewed 14 research studies in which different methods were employed to repress neurogenesis, such as X-ray radiation, cell proliferation inhibitor (methylazoxymethanol acetate), and genetic manipulation followed by cognitive testing.[45] Five of the fourteen experimental studies revealed cognitive dysfunction after the inhibition of neurogenesis, while eight did not and in one there was an effect on learning in Barnes maze but not water maze. In eight experiments there was a deficiency in short-term memory and long-term memory in three without the deficiency. These results were inconsistent and it remained unclear whether neurogenesis, learning and memory were causally linked. Additionally, other studies revealed that neurogenesis played an important role in specific hippocampal-dependent learning and memory process, such as pattern separation, the demands for the ability of pattern separation in different experimental methods and instrumentation and varied tasks which likely accounted for the inconsistencies. It is therefore concluded that learning and memory was constrained when neurogenesis was prevented. In addition, it was reported that the increase of hippocampal neurogenesis improved pattern separation ability.

Lesions in the hippocampus impaired learning and memory in the touchscreen training and study.[46] However, Jaholkowski *et.al.* discovered that cyclinD2 knockdown could inhibit neurogenesis of the mouse, whereas, the mouse is still capable of learning. Thus, they proposed that neurogenesis was not indispensable for learning and memory, nevertheless, they suggested that the role of neurogenesis in promoting learning and memory could not be neglected. [47, 48] Clelland *et. al.* proposed a critical threshold relating neurogenesis with learning and memory as they found similar impairment of pattern separation between mouse with 50% decline in neurogenesis and those with a total deletion of neurogenesis.[49]

It has been proposed that a slight reduction in hippocampal neurogenesis had no influence on the memory ability of animals. However, when there was a significant reduction in hippocampal neurogenesis, the hippocampal-dependent learning and memory would be impaired. Even a short period of hippocampal neurogenesis in the early adulthood was sufficient to optimize the hippocampal neural networks and affect the fixated behavior during the whole lifespan.[37]

Questions have been then raised as to whether most of the message processing of DG were accomplished by the new-born neuron, why could such a small amount of the new-born neuron influenced the function of the whole brain. Further studies revealed that the expression of immediate early gene regulation of the activation of adult-born granule cells could be important biomarkers. Since new-born neurons in the hippocampus were of higher excitability, they were more sensitive to input signal, less affected by GABA neurotransmitter and thus more easily activated.[50] Additionally, new-born neurons could integrate into the hippocampal neural networks rapidly, competing with the mature neuron thus generating action potentials at a threshold ten times less than the adult granule cells. This effectively facilitated the input and process of the message,[5] participated in the induction and maintenance of LTP and promoted the hippocampal-dependent learning and memory.[51] New-born neurons could be correlated with many different intermediary neurons and regulate the function of other brain regions by selectively activating several pathways [52]. Therefore, a small amount of the new-born neuron population could influence the function of the whole brain. Taken together, we conclude that hippocampal-dependent learning and memory and neurogenesis are mutually promoted. However, whether visual perceptual learning tasks can improve the function of hippocampus by regulating of hippocampal neurogenesis requires further investigation.

BDNF signal pathway and learning and memory

BDNF, a member of the neurotrophin family, is highly expressed and widely distributed in various regions of the brain, such as the hippocampus, striatum, amygdala, cortex and cerebellum. It could be secreted in a target, autocrine or paracrine manner and exert its effect on neural function through tyrosine kinase (TrkB) or p75 neurotrophin receptor (p75NTR) by activating signal pathways such as RAS-MEK (MAPK and ERK), PI3K-Akt, PLC-γ-IP3 /DAG. [53, 54] A moderate dose of BDNF promotes learning and memory, although BDNF disturbs the balance between inhibitory and irritating neurotransmitter and exerts a negative role in learning and memory when there was too much or too little BDNF.[55] Interestingly, the effect of BDNF on memory is specific to different stages of memory, as the coding, storage, and consolidation of pattern separation memory is BDNF-dependent, while the retrieval of memory is not BDNF-dependent.[56] Further research revealed different mechanism underlying short term memory and long term memory. For short term memory, BDNF regulates synaptic transmission quickly by phosphorylation of synaptic protein. For

example, BDNF promotes phosphorylation of presynaptic synapsin I by MARK and facilitates the transfer and release of synaptic vesicles, the extension and maintenance of axon, and signal communication between synapses[43] and promotes phosphorylation of RIM1a by ERK2 and Rab3 signaling and facilitates neural depolarization and the release of glutamate.[57] BDNF phosphorylation and activation of the postsynaptic NMDA and AMPA receptors, increases the postsynaptic response to glutamic acid. On the other hand, BDNF facilitates formation and storage of long-term memory by regulating the expression of synaptic proteins, ribosomal proteins, channel proteins and cytoskeleton proteins involved in long term memory.[58]

Given the critical role of BDNF in learning and memory, it is of interest to examine the regulation of BDNF and how it enhances learning and memory. As BDNF has complex structure, its expression is regulated during transcription, translation, and post-translation by various molecular, one of which is cAMP-responsive element binding protein, an important regulator of BDNF and could, in turn, be regulated by BDNF, forming the positive feedback of self-regulation, thereby improving learning and memory.[59] Additionally, BDNF is secreted in a "as need" manner, as it is only elevated only when the DG separating similar pattern, but the pattern is dissimilar, [56] and the mechanism has yet to be explored.

Regulation of BDNF by learning and memory BDNF promotes learning and memory and learning increases the mRNA and protein expression of BDNF. Berchtold *et al.* has observed that the expression of BDNF in the hippocampus of mouse was significantly increased by 186% one week after the task of radial six-arm water maze, then began to fall two weeks later, and returned to baseline 3 to 4 weeks after the task. At the same time, the exploring and memory ability was detected and showed it to be best immediately after the task and one week after the task suggesting that the dynamic change of the BDNF level was closely related with cognition. [60] Alomari *et al.* reported that both forced and voluntary exercises could improve spatial learning as well as the expression of BDNF, TrkB, and the downstream signaling molecules such as Akt, GSK-3β, mTOR, and p70S6K. [61, 62] Resistance exercise also enhanced cognitive function in mouse associated with increased expression of BDNF and CREB in the hippocampus.[63] In addition, rats with 2-vessel occlusion treatment, an experimental model of chronic cerebral hypoperfusion, exhibited the pathophysiological features of human Alzheimer's disease and vascular dementia, whereas, environmental enrichmentment could restore cognition depending on the promotion of hippocampal synaptic plasticity and the expression of BDNF.[64]

Extensive studies have been performed on the relationship between BDNF, hippocampal neurogenesis and learning and memory. However, whether touchscreen training affects hippocampal neurogenesis and learning and memory through the signal transduction pathways mediated by BDNF is yet to be determined.

Regulation of hippocampal plasticity by BDNF Hippocampal plasticity is important in learning and memory, therefore, it is important to explore whether BDNF improves learning and memory through regulating hippocampal plasticity. It has been noted that BDNF regulates the structure and function of the neuron throughout life. In immature neurons, BDNF is involved in the survival, proliferation, differentiation, maturation, and migration of new-born neurons [65-67] and promotion of neurogenesis .[68] In mature neurons, BDNF plays an important role in synaptic plasticity and participates in the structure and function of the synapse and increases synaptic density and dendritic arborization in the hippocampus[69, 70], enhance neurotransmission and receptor sensitivity [71, 72] and consolidate memory[73]. Thus, BDNF exerts a specific coupling effect between neural structure and learning and memory. BDNF is considered a "gatekeeper" for the storage and regulation of memory, important for cognitive function.

Conclusions

In addition to the synaptic plasticity which is widely regarded to be conductive to the plasticity of the hippocampus, hippocampal neurogenesis represents a form of cellular plasticity of hippocampus, which is essential for learning and memory. Learning, environmental enrichment and exercise could promote neurogenesis, which in turn, improves hippocampal-dependent learning and memory and even rescue learning and memory dysfunction resulted from the reduced hippocampal neurogenesis. Therefore, learning exercise and hippocampal neurogenesis are mutually promoted. The importance of BDNF-mediated hippocampal neurogenesis and learning and memory through the regulation of relevant gene transcription and protein translation by several signal pathways has been illuminated. As a special way of learning, touchscreen visual perceptual training can enhance memory, thereby providing a new method for cognition research. However, further exploration are required in the future: What is the quantitative-effect relationship between the maintenances of the learning and memory and the plasticity of NSCs? Which molecular pathway is critical for the regulation of neurogenesis? Finally, how to optimize the touchscreen visual stimulation, as a non-invasive method, to facilitated neurogenesis and signal transduction, so as to improve learning and

memory?

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Key Messages

Touchscreen visual perceptual training is a novel way of behavioral test.

Learning exercise, neurogenesis and learning & memory are mutually promoted.

BDNF mediated hippocampal neurogenesis and learning & memory.

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