Research Report

Running Improves Pattern Separation during Novel Object Recognition

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Abstract. Running increases adult neurogenesis and improves pattern separation in various memory tasks including context fear conditioning or touch-screen based spatial learning. However, it is unknown whether pattern separation is improved in spontaneous behavior, not emotionally biased by positive or negative reinforcement. Here we investigated the effect of voluntary running on pattern separation during novel object recognition in mice using relatively similar or substantially different objects. We show that running increases hippocampal neurogenesis but does not affect object recognition memory with 1.5 h delay after sample phase. By contrast, at 24 h delay, running significantly improves recognition memory for similar objects, whereas highly different objects can be distinguished by both, running and sedentary mice. These data show that physical exercise improves pattern separation, independent of negative or positive reinforcement. In sedentary mice there is a pronounced temporal gradient for remembering object details. In running mice, however, increased neurogenesis improves hippocampal coding and temporally preserves distinction of novel objects from familiar ones.

Keywords: Adult neurogenesis, hippocampus, granule cell dendrites, novel object recognition, learning and memory

It is well known that the dentate gyrus is critically important for pattern separation within the hippocampal network [1, 2]. Furthermore, it was shown, that adult neurogenesis in the dentate supports pattern separation during hippocampus-dependent memory tasks. This notion is based on the observation that ablation of adult neurogenesis disrupts distinction of similar memories and disturbs differential population coding of similar memory items in the hippocampal CA3 network [3–5]. For example, animals with intact adult neurogenesis can distinguish similar context during context fear conditioning, closely spaced items on a spatial touch screen or neighboring arms in an 8-arm radial maze [6–8]. After ablation of adult neurogenesis, however, similar items cannot be distinguished anymore, whereas distinct items, like distinct context, can still be remembered by the animals. Furthermore, newly generated neurons are particularly important during the first 4 weeks after mitosis, as pattern separation in context fear conditioning is most sensitive to manipulations targeted to this young population of granule cells [9, 10]. Interestingly, this time period largely overlaps with a critical period for enhanced synaptic plasticity and synaptic integration of the newly generated young neurons into the hippocampal circuitry [11–13]. The enhanced plasticity contributes to neuronal pattern separation, as blocking synaptic plasticity in a cohort of newly generated young granule cells (>6 weeks post mitosis) by genetic deletion of NR2B receptors strongly reduced context discrimination after context fear conditioning [14].
Physical exercise was reported to increase hippocampal stem cell proliferation and adult neurogenesis [15]. As a consequence hippocampus-dependent learning and memory formation is improved by voluntary wheel running in mice [16, 17]. In particular, pattern separation in a touch screen task was shown to be more precise in running mice as compared to sedentary animals [18]. Remarkably, all the behavioral tests assessing pattern separation used behavioral tasks involving positive (food reward) or negative (electric shock) reinforcement strategies to generate detectable behavioral output. The important contribution of emotion would be consistent with anatomical data, showing extensive hippocampal connectivity with subcortical structures like dorsal raphe, VTA, locus coeruleus, amygdala and nucleus accumbens [19]. Therefore, it is unclear whether improvement in pattern separation is restricted to memory items which are emotionally charged, or whether it generally applies for hippocampus-dependent learning tasks.

For example, it is unknown, whether pattern separation in spontaneous behavior such as Novel Object Recognition (NOR) is also affected by changes in adult neurogenesis. This test is based on the spontaneous tendency of mice to preferentially explore a novel, previously unknown object relative to familiar objects. Thus, no reward or punishment is necessary to be associated with the behavior and the emotional content is minimal. The effect of increasing or decreasing adult neurogenesis on NOR is controversial. Whereas some studies could not detect any impairment in NOR memory after reduction of adult neurogenesis [20–22], there was a disruption of NOR memory reported by others [14, 23, 24].

To test effects of physical exercise on pattern separation in a less emotional task, we used a NOR paradigm with different types of objects. We analyzed exploration time of objects which were either similar or very distinct to familiar sample objects. Animals with free access to running wheels were compared to sedentary control animals, showing significant differences in the recognition of similar objects but not distinct objects. This indicates that running significantly improves pattern separation even if the emotional charge is minimal.

MATERIALS AND METHODS

Animals and housing conditions

Female 8-week-old C57BL/6 mice were purchased from Harlan Laboratories (Switzerland). Animals were placed for 3 weeks into different housing conditions using a 12:12 h light/dark cycle. Control mice were kept in eurostandard type II cages (267 × 207 × 140 mm), three animals per cage. Mice with free access to a running wheel (RW) were kept either in eurostandard type III cages (RW1, 425 × 266 × 185 mm) or environmentally enriched eurostandard type IV cages (RW2, 595 × 380 × 200 mm), with three or six animals per cage, respectively. All animals received some tissue paper to allow nest building. The behavioural studies were performed when the animals were ∼11 weeks old. The experiments were approved by the animal advisory committee of the Kanton Basel (Kantonales Veterinäramt BS, Switzerland).

Novel object recognition

To assess learning and long-term memory a Novel Object Recognition (NOR) task was used. Two identical objects were placed into the arena during a 6 min sample phase. Subsequently, one of the objects was exchanged by a new object and memory was assessed by comparing the time spent exploring the novel object as compared with the time spent exploring the familiar object during a 5 min test phase.

One week before the NOR experiments, the animals experienced handling by the experimenter and habituation to the arena for 2 and 3 consecutive days, respectively. The handling procedure included exposure to a transparent plexiglas tunnel with a length of 12 cm and a diameter of 6 cm. Using this tunnel, an animal was transferred into a new cage and after a few minutes back again to home cage. This type of handling apparently reduced anxiety as described previously [25]. Animals which were still jumping in the NOR test were excluded from the analysis (8 out of 59 mice). For habituation, mice were placed into the empty arena (38 × 38 × 30 cm, PVC) for 5 min. During all experiments the arena was illuminated with 60–90 Lux.

For NOR experiments custom-built plastic pieces (Polyoxymethylene, POM), were used with different shapes (cones: 4 cm diameter, 6 cm height, pyramids: 4 × 4 × 4–6 cm) and different color (white, black, red). The objects were cleaned thoroughly with 40% ethanol followed by distilled water between trials to remove olfactory cues. During the sample phase on the first day of the NOR test, the mice were allowed to explore the two identical white or black objects (either two cones or two pyramids) for 6 min. For the short-delay test phase (1.5 h) one of the sample objects was replaced by a new one (cone by pyramid or vice versa) and exploration
was measured for 5 min. For the long-delay test phase (24 h) the new object was again exchanged by another new object. The location of the novel object at 24 h was always different from that at 1.5 h, either first left then right, or vice versa. Consequently, the location of the familiar object also switched between the two test phases. Objects with the same color but different shapes were considered to be similar to sample object. Objects with both, different shape and different colour (red, black, white) were considered to be distinct from the original sample objects.

The behaviour of the animals was recorded with a Logitech HD Pro Webcam C920 and running tracks of the animals during NOR were traced with Videomot2 (TSE Systems, Germany). To analyse strictly active exploration the time was measured manually using a digital stopwatch (Silva, Sweden). Active exploration was defined as direct sniffing or whisking towards the objects or direct nose contact. Climbing over the objects was not counted as exploration. The relative exploration the time was measured manually using a digital stopwatch (Silva, Sweden). Active exploration was defined as direct sniffing or whisking towards the objects or direct nose contact. Climbing over the objects was not counted as exploration. The relative exploration was quantified by normalizing the difference between the exploration time of the novel (Tn) and familiar object (Tf) by the total time of exploration (Ttot) to calculate the NOR discrimination index: NOR index = (Tn–Tf)/Ttot. With identical sample objects the NOR index was always less than 0.2 (average = 0.07, ±0.15, n=6, p=0.571).

Immunohistochemistry

Three to four days after behavioral testing, the animals were anesthetized with isoflurane (4% in O2, Vapor, Draeger) and killed by decapitation, in accordance with national and institutional guidelines. Transverse hippocampal brain slices were cut in a sucrose-based solution using a Leica VT1200 vibratome (26) and slices were fixed overnight with 4% paraformaldehyde at 4°C. After washing with PBS, tissue sections were incubated overnight at 4°C with the primary antibody Goat-anti-DCX (1:200–500, c-18, sc-8066, Santa Cruz, IgG) in PBS including 0.3% Triton X-100 and 5% Normal Donkey Serum. The secondary antibody Donkey-anti-goat Alexa 488 (1:500, A11055, Molecular Probes, Invitrogen) and DAPI (1:10000, Roche) were applied at the second day in PBS with 0.3% Triton X-100 and kept overnight by 4°C. The following day after washing, the slices were embedded in Prolong Gold (Molecular Probes, Invitrogen).

Fluorescence microscopy and image analysis

Fluorescence images were acquired using a Zeiss LSM 700 confocal microscope. For counting DCX+ cells in the hippocampus we used a 20 × objective (NA 0.75). To measure the number of DCX+ cells in different animals, we first counted the number of DCX+ cells within 18 μm thick confocal sections, which were obtained in all cut slices from one hemisphere at a depth of 10 μm below the surface. DCX+ cells were counted manually in one optical section per slice with ImageJ using the plugin Cellcounter. For analysis of density of DCX+ cells in dorsal and ventral sub-regions, the number of cells per section was normalized by the length of the granule cell layer measured in the DAPI staining.

To obtain the total number of cells per hemisphere, the number of cells in 3-μm sections was extrapolated to the number of cells in the 3D volume of the 130 μm thick slices. First, we performed control experiments, counting all DCX+ cells in the 3D volume of 45 μm thick confocal sections (n = 4) by using a grid-based analysis in ImageJ. Each stack consisted of 30 sections with a thickness of 3 μm obtained at an interval of 1.5 μm. We then compared this 3D-cell number with the number of cells visible in 15 non-overlapping, adjacent 3-μm confocal sections of the corresponding stack. The ratio of these two numbers was 1.65 ± 0.15 (n = 4), indicating that a single cell is on average visible on 1.65 adjacent 3-μm sections. Second, we considered the thickness of embedded slices (150 μm), the thickness of a single confocal section (3 μm) and the fact that a single cell is visible on 1.65 adjacent sections to calculate a scaling factor f = 100 μm/(3 μm × 1.65) = 30.3. Finally, we multiplied the number of DCX+ cells per section in each slice with this scaling factor to extrapolate from section to slice and to calculate the sum of all DCX+ cells per hemisphere. For example, a number of 405 cells counted in imaged sections, thus leads to 12 272 DCX+ cells per dentate.

This method allowed to use thick slices, to preserve the dendritic tree of cells for further analysis (see below). Furthermore, as f was the same for all slices and for all animals, the relative comparison between animals in the different cohorts was independent of this stereological simplification.

For analysis of dendrites, image stacks were acquired with a 40 × oil-immersion objective (NA 1.4). 20–50
images were taken at an interval of 0.5 μm with a slice thickness of 1 μm. Maximum intensity projections were calculated and dendrites were counted in the inner (IML) and outer molecular layer (OML) at a relative distance from the granule cell layer of 15% and 50% of the total molecular layer, respectively. In addition to directly counting the number of dendrites, we determined DCX fluorescence as an independent measure of dendrite outgrowth. The average background-subtracted fluorescence intensity was measured within rectangular areas of about 40 × 250 μm in the IML close to the granule cell layer, as well as in the middle of the OML. Background intensity was measured from areas devoid of DCX-positive dendrites in the outer border of the OML. For analysis of dorsal versus ventral hippocampus, data from the dorsal and ventral half of the slices were pooled and displayed separately.

Statistics and data presentation

All data are presented as mean ± SEM. All statistical analysis was done with GraphPad Prism6. For all comparisons, a two-tailed Wilcoxon-Mann-Whitney Test was used. The significance level was set to \( P < 0.05 \).

RESULTS

Running increases number and dendritic length of newly generated young neurons

To study the impact of voluntary running on learning and memory, adult 8-week-old female mice were housed in different conditions for 2-3 weeks prior to behavioral analysis (Fig. 1). To analyze the impact of running wheels on adult neurogenesis, young granule cells were stained with an antibody against doublecortin (DCX) [27]. Voluntary running significantly increased the number of DCX+ young granule cells (Fig. 2C, 15 593 ± 663, \( n = 8 \)) relative to control (11 506 ± 500, \( n = 8 \), \( P < 0.05 \)). The effect was most pronounced in the dorsal hippocampus with an about 2-fold increase in the number of young neurons (Fig. 2D, E). Furthermore, we analyzed the dendritic growth by counting dendrites in the inner (IML) and outer molecular layer (OML) of the dentate gyrus. In sedentary animals, most of the DCX+ dendrites grow...
As shown in Fig. 2F, the dendrites of young granule cells are longer with more dendrites in the outer molecular layer, where fibers from the entorhinal cortex terminate (Fig. 2B). As the number of DCX+ neurons was larger with running wheels, we normalized the number of long dendrites by calculating the ratio of the number of OML (50%) to IML (15%) dendrites (Fig. 2A, B). As shown in Fig. 2F, the dendrites of young granule cells are on average longer with running wheels, with 34±4% of the dendrites extending well into the OML, significantly more than in control animals (21±4%, P<0.05, n=10 each). Again this effect was largely constrained to the dorsal half of the hippocampus showing 3 times more dendrites in the OML in running animals (Fig. 2G, 34±5% versus 11±4% in control, P=0.0038, n=7 and n=5, respectively). Similarly, calculating the ratio of DCX-fluorescence in the OML relative to the fluorescence in the IML revealed a 2-fold increase in dorsal DCX fluorescence with running (61±10% versus 34±6%, P=0.0294, n=11 and n=10, respectively, Fig. 2H). The running-induced increase in dendritic outgrowth is consistent with a recent report, showing that dendritic growth in newly generated 2-week-old granule cells is accelerated by exercise [28]. Together with the 2-times larger number of young granule cells in running mice in the dorsal hippocampus, this corresponds to a 4-fold increase in number of young GC dendrites in the molecular layer. As a consequence there might be a 4-times larger chance for the formation of new synapses of young cells with axon terminals projecting from the entorhinal cortex.

Taken together, the data not only show that the animals make use of running wheels, leading to the well-known increase in the number of newly generated granule cells. It further strengthens the notion that also dendritic morphology is changed by wheel running with more dendrites in the termination zone of entorhinal input fibers.

### Running improves pattern separation during novel object recognition

To study hippocampus-dependent learning and memory we used a novel object recognition task with a 6 min sample phase, followed by a 5 min test phase either 1.5 h (Fig. 3) or 24 h later (Fig. 4). In the sample phase animals were always exposed to two identical objects, either cones or pyramids with the same color (Fig. 3B). During the sample phase animals used on average 29.5±1.4 s (n=45) for active object exploration. The exploration time of the two identical objects was very similar leading to a NOR-discrimination index of –0.02±0.02 in control (n=20) and 0.00±0.02 with running wheels (n=25, Fig. 3A). This suggests that the animals do not show a bias towards the right or left side. Exchanging one of the objects by a novel object, however, induced a significant side preference towards the novel object in the 1.5 h-delay test phase. This was independent of whether the new object was similar to (Fig. 3C, D) or distinct from the sample object (Fig. 3E, F). Using a similar object with the same color (but different shape) the running animals showed a discrimination index of 0.18±0.07 (n=9) comparable to control animals (0.15±0.06, n=9, P=0.932). Similar results were obtained with distinct objects having both, different color and different shape for running (0.21±0.04, n=16) and sedentary mice (0.23±0.08, n=11, P=0.846). This shows that the animals are perfectly able to distinguish sample objects from similar test objects 1.5 h after the sample phase, independent of enhanced adult neurogenesis.

Furthermore, we tested the animals at a later time point 24 h after the sample phase presenting again the familiar object as well as a further novel object, with switched locations relative to the 1.5 h test (Fig. 4). Animals with previously similar objects at 1.5 h delay were exposed to a novel distinct object at 24 h delay (Fig. 4A, B), whereas animals with previously distinct objects were exposed to a novel object similar to the sample (Fig. 4C, D).

During this long-term retention test, there was a significant difference between running and control mice. With distinct objects, sedentary mice are able to remember which of the objects is familiar and which is novel, leading to a discrimination index of 0.21±0.06 (n=9) comparable to running mice (0.30±0.05, n=9, p=0.398). With similar novel objects, however, the running animals remembered the familiar object significantly better (0.21±0.04, n=16) than sedentary mice (0.07±0.05, n=11, p<0.05). This difference is further exemplified in Fig. 4E, F showing representative 2 min example tracks of a control (Fig. 4E) and a runner mouse (Fig. 4F) during the 24 h-delay NOR test. The differential recognition of similar and distinct objects clearly shows that the control animals remember the familiar sample object 24 h after the sample exposure. However, the memory trace apparently contains less detail as compared to the runner mice with enhanced neurogenesis, indicating that there is a difference in neuronal pattern separation.
Exercise inhibits temporal decay of pattern separation after learning

To compare differences between the 1.5 h-delay and the 24 h-delay NOR test we plotted the discrimination index against memory retention time (Fig. 5). Whereas both, runners and control animals show a similar discrimination index for distinct objects of about 0.2 at 1.5 h and 24 h (Fig. 5A) after learning, there is a pronounced temporal gradient for the retention with similar objects in control but not in running mice (Fig. 5B). The clear distinction of similar objects at 1.5 h in control mice suggests that there is no difference in sensory perception of details. Also it seems that details can be transiently encoded and retrieved 1.5 h after the sample phase, suggesting no difference in short-term memory. However, in control animals, the recognition memory clearly decays within the next 24 h with a NOR index close to chance level. By contrast, this decay is prevented in running mice, potentially by an increased number of newly formed synapses in the OML with 4-times more young dendrites in running mice.

We also compared memory retention in mice housing with only a running wheel (RW1) to mice with additional enrichment with tunnels and small houses (RW2), which did not lead to significant differences in the NOR index at 1.5 h and 24 h delay (Fig. 5C, D). The temporal decay in pattern separation is prevented with both paradigms. This suggests that the most important factor for improving pattern separation in our study is physical exercise by voluntary wheel running.
Fig. 4. Running improves pattern separation and recognition memory at 24 h delay. A, B. After 24 h delay, one sample object (cone) was replaced by a distinct object (red pyramid) shown in (B). Animals spent significantly more time in exploring the novel object relative to the familiar object in both, control (n = 9) and running mice (n = 9). C, D. In a different cohort of animals one sample object (cone) was replaced by a similar object (white pyramid) shown in (D)). At 24 h delay, running animals spent significantly more time in exploring the novel object (n = 11) relative to control animals (n = 16). Control animals did not show a significant recognition memory for the familiar object at 24 h delay. E, F. Example tracks showing a 2 min path of a control animal (E) and a running animal (F), during the 24 h delay novel object recognition test. The novel object in E and F was placed on the left.

Running. This would be consistent with previous findings, showing that enhanced physical exercise is the most powerful neurogenic factor of environmental enrichment [29, 30].

Taken together, these data indicate that physical exercise improves pattern separation by preserving the hippocampal processing of memory details over time, which might decay rapidly otherwise.

DISCUSSION

We have studied the effect of voluntary running on adult neurogenesis, dendritic growth and learning...
behavior. Using NOR, we show that running improves pattern separation in a learning task, which does not involve negative or positive reinforcement strategies. This conclusion is based on several important findings. First, testing NOR memory at 24 h after the sample phase shows that only running mice could distinguish familiar objects from similar novel objects, whereas sedentary animals could not. Second, running did not affect NOR memory of highly distinct objects, indicating that sedentary animals still remember the familiar object 24 h after sample phase, although less precise. Third, similar objects could be clearly distinguished by sedentary mice with a 1.5 h delay, showing that perception of details and short-term memory was not affected by running. Thus, the data show that running improves hippocampal pattern separation during spontaneous behavior, to generate unique and detailed long-term representations of similar but nevertheless different memory items.

Running-induced increase in hippocampal neurogenesis and dendritic growth

It is well known that physical exercise like voluntary running in running wheels substantially increases proliferation of adult neural stem cells leading to increased adult hippocampal neurogenesis [15, 29, 30]. However, the dorso-ventral distribution was only rarely investigated. We have found that running wheels preferentially increase neurogenesis in the dorsal hippocampus. A similar preferential increase in adult neurogenesis in dorsal hippocampus was also shown previously [31]. Conversely, chronic mild stress and fluoxetine, selectively decrease and increase neurogenesis in the ventral hippocampus, respectively [32]. What are the mechanisms underlying the running-induced neurogenesis in the dorsal hippocampus? Physical exercise increases BDNF release in the hippocampus in a dose dependent manner [33]. Adult hippocampal stem cells express TrkB receptors, which
are activated by NMDA-receptor dependent BDNF release from mature neurons [34]. Mice with genetic deletion of NR2A-NMDA receptors neither show exercise-induced BDNF-release nor running-induced increase in neurogenesis [35]. Furthermore, CamKII-Cre-dependent conditional knock-out of BDNF or deletion of TrkB-receptors in GFAP+ hippocampal stem cells abolished running-induced increase in proliferation and neurogenesis [36, 37]. Finally, wheel running stimulates excitatory synaptic inputs from the entorhinal cortex and induce hippocampal synaptic plasticity [38, 39]. So far, this wheel-running-induced excitation was measured only in the dorsal hippocampus. However, running in an open field induces theta oscillations with about 10-fold smaller amplitudes in ventral as compared to dorsal hippocampus [40]. Thus, the preferential stimulation of dorsal hippocampal neurogenesis could be due to a potentially lower activation of the ventral hippocampus due to different grid-cell activity and other functional differences along the dorso-ventral axes [19].

In addition to the increased number of neurons we found increased dendritic growth of DCX+ young granule cells leading to about 2- to 3-times more dendrites per cell extending into the OML of the dorsal dentate gyrus. This is consistent with Golgi-impregnation studies showing an increased dendritic length in granule cells and other hippocampal neurons in running mice [41, 42]. However, our data together with a recent study showing that running specifically accelerates dendritic growth in 2-week-old immature neurons [28], indicates that young granule cell dendrites are most sensitive. The enhanced dendritic growth could be mediated by running induced activation of GABAergic and glutamatergic synapses, which were shown to regulate dendritic growth [43–45]. By contrast, dendrites of 4-week-old cells are not affected anymore by exercise [28]. In contrast to dorsal hippocampus, we could not find enhanced dendritic outgrowth in ventral regions. However, this does not exclude running-induced effects on spine formation or synapse formation, which were reported in ventral hippocampus by others [46, 47].

Overall, this activity-dependent increase in proliferation and dendritic growth leads to a 4-fold increase of young dendrites in the OML, where entorhinal axons terminate. These new dendrites can exquisitely compete for new synapses with preexisting granule cells [48], and may account for the pronounced enhancement of long-term potentiation (LTP) observed in dentate gyrus of running mice [16, 49].

Running increases pattern separation during NOR

Using similar objects we have found a strong effect of running on NOR memory at 24 h delay after sample phase. As running specifically increased adult neurogenesis in the dorsal hippocampus, this would be consistent with a previous report showing that NOR memory is disrupted after lesion of the dorsal dentate gyrus [50]. The data are also consistent with several studies showing running improves NOR memory [32, 51–54]. Conversely, disruption of NOR memory was reported after ablation of adult neurogenesis by antimitotic agents or X-ray irradiation [23, 24] as well as after inhibition of synaptic plasticity specifically in the newly generated neurons younger than 6 weeks post mitosis [14]. However, these findings are in contradiction with other previous studies claiming that the reduction of adult neurogenesis does not affect NOR memory [20, 21, 55].

Our data provide a potential solution for this apparent discrepancy, as we have found that the effects of running on NOR memory is dependent on the relative similarity of the used objects. The difference we have found between similar and distinct objects at 24 h suggests that the sedentary animals do not remember exactly the shape of objects they have seen one day before. Therefore, they do not consider the similar novel object as novel. By contrast, with very different objects NOR memory is comparable to running mice and therefore probably independent of adult neurogenesis. Only if objects are relatively similar, young neurons may specifically contribute to pattern separation during NOR, similar to what was shown for other hippocampus-dependent tasks as for example navigation in an 8-arm maze or context fear conditioning [6, 7, 9]. Therefore, the usage of different types of objects would explain the contradictory results of the different NOR studies mentioned above.

We have found a strong effect of running on pattern separation during NOR memory at 24 h delay. However, with 1.5 h delay there was no significant difference in control animals compared to running mice. Sedentary animals could nicely distinguish the novel objects, which were relatively similar to familiar objects. A temporal gradient of running/enrichment dependent enhancement of NOR memory was also observed in other studies [23, 52]. The fact that memory retention at about 1 h after sample phase is not affected by running (our study) and after 50% decrease of neurogenesis via antimitogenic agents [23] would indicate that neurogenesis is not required for short term...
Contribution of enhanced neurogenesis to running induced increase in pattern separation during NOR

Although running induces both, substantial changes in number and morphology of young neurons as well as significant changes in learning behavior, this does not prove a causal relationship. Instead, these two phenomena could occur in parallel without direct functional interconnection. Nevertheless, there are several arguments, suggesting that increased adult neurogenesis during running indeed contributes to the enhanced pattern separation during NOR.

First, our data indicate that long-term memory was improved at 24 h without an obvious change in sensory processing because of (similar NOR at) 1.5 h, pointing towards a change in hippocampal function. Second, acquisition of NOR memory was shown to be strongly reduced after blocking synaptic plasticity specifically in a cohort of newly generated young granule cells (<6 weeks post mitosis) by genetic deletion of NR2B receptors [14]. Third, newly generated granule cells contribute to pattern separation in context fear conditioning, as context discrimination is decreased after inhibition of hippocampal proliferation [9] or after functionally silencing a population of young neurons [10]. Finally, DCX+ positive young granule cells are critically important for consolidation of object memories, as NOR at 24 h delay was disrupted by ablation of DCX+ neurons 1 h after sample phase [24]. Taken together, these data converge to the notion that increased neurogenesis may significantly contribute to running-induced improvement of pattern separation during NOR.

The mechanisms underlying the neurogenesis-dependent consolidation of object memories are largely unclear. The available data would indicate that young neurons probably form new synapses during learning with competitive advantage relative to pre-existing mature cells [48]. However, this might not directly impact on learning behavior. The main associative memory-storage unit in the hippocampus is the CA3 network, which receives direct inputs from the entorhinal cortex and rapidly adjusts recurrent synaptic connections in an activity-dependent manner [2, 9, 56]. However, temporal stability of synaptic plasticity like long-term potentiation (LTP) is known to be activity dependent and weak stimuli induce LTP, which decays back to baseline within a few hours. Granule cells not only form powerful excitatory mossy-fiber synapses onto CA3 pyramidal cells, but even more numerous, synapses with GABAergic interneurons generating efficient feed-forward inhibition in CA3 [57, 58]. Inhibitory mossy-fiber synapses as well as feed-forward inhibition are well developed already at 4 weeks after mitosis, much earlier than feedback inhibition [10, 59]. As a consequence, newly generated granule cells might increase firing in some, but decrease firing in other CA3 pyramidal cells, thereby enhancing contrast between the activity of different cells, to shape population coding of similar objects within the CA3 network [5].

Together, these findings would indicate that the increased number and dendritic growth of young neurons after running contribute to increased activation of a ‘specific’ set of CA3 pyramidal cells, to induce long-lasting late LTP in distinct CA3 cell assemblies, coding for similar but nevertheless distinct memory items. Without synaptic inputs from young granule cells, synapses in CA3 cell assemblies are less specific and less strongly activated. Thus early LTP decays back to baseline with time, generating a degraded representation of memories. Therefore, exercise-induced increase in neurogenesis improves pattern separation by supporting unique and detailed long-term representations of similar but nevertheless different memory items to finally preserve hippocampal processing of memory details over time.

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