Regular and moderate exercise initiated in middle age prevents age-related amyloidogenesis and preserves synaptic and neuroprotective signaling in mouse brain cortex

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ABSTRACT

Although the beneficial responses induced in the central nervous system by early-initiated exercise have been broadly investigated, the effects of a chronic and moderate lately-initiated exercise on biochemical hallmarks of very early brain senescence have not been extensively studied. We previously reported that a midlife-initiated regimen of moderate running was able not only to prevent the age-related decay of antioxidative and detoxification functions in mouse brain cortex, but also to preserve neurotrophic support and molecular integrity. On this basis, this work investigated whether and how a 2-mo or 4-mo midlife-initiated running protocol could affect the activity of those systems involved in maintaining neuronal function and in preventing the onset of neurodegeneration within the brain cortex of middle-aged CD-1 mice. In particular, we analyzed the production of the peptide amyloid-β and the expression of synapsin Ia, which is known to play a key role in neurotransmission and synaptic plasticity. In addition, we studied the expression of sirtuin 3, as a protein marker of neuroprotection against age-dependent mitochondrial dysfunction, as well as the pro-death pathway induced by proBDNF through the interaction with p75NTR and the co-receptor sortilin.

The midlife-initiated 4-mo running program triggered multiple responses within the mouse brain cortex, through the activation of anti-amyloidogenic, pro-survival, synaptogenic and neuroprotective pathways. However, most of the beneficial actions of the exercise regimen appeared only after 4 months, since 2-mo-exercised mice showed marked impairments of the endpoints we considered. This could imply that a midlife-initiated regimen of moderate treadmill running may require an adequate time lag to activate beneficial compensatory mechanisms within the mouse brain cortex.

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1. Introduction

A healthy lifestyle improves mental activity and lowers the risk of several diseases. In the central nervous system (CNS), neural repair and synaptic plasticity can be enhanced through some non-pharmacological interventions (Mattson, 2012). Human and animal studies have suggested that exercise promotes neuroplasticity and improves cognitive functions (Ahlskog et al., 2011; Ferreira-Vieira et al., 2014; Gomez-Pinilla and Hillman, 2013). Adaptive responses induced in the CNS by early-initiated exercise have been broadly investigated, whereas the effects of regular and moderate lately-initiated exercise on biochemical hallmarks of very early brain senescence have not been extensively studied (see Camiletti-Moirón et al., 2013; Radak et al., 2008a).

Some population-based studies have shown a reduced risk of dementia in the elderly undergoing physical activity in the middle age (Andel et al., 2008; Geda et al., 2010), but most of the molecular players involved in such beneficial effects in the mammal CNS are still unknown.

We have recently demonstrated that a long-term regimen of midlife-initiated moderate running prevents the age-related decay of brain antioxidative and detoxification functions, and preserves both neurotrophic support and molecular integrity (Falone et al., 2012a, 2012b), thus supporting other findings about the positive effect of exercise on neuroprotection (Leite et al., 2012) and antioxidant capacity (Cechetti et al., 2008, 2012).

In this work we investigated further the influence of middle age-initiated exercise on molecular pathways which are particularly prone to age-related decline. In particular, we studied whether and how a long-term midlife-initiated enforced running protocol could affect the activity of those systems involved in maintaining neuronal health and

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in preventing the onset of neurodegeneration within CD-1 mouse brain cortices.

The peptide amyloid-β (Aβ) is implicated as a central factor in neurodegenerative processes since Aβ accumulation leads to neuronal apoptosis, synaptic loss and glial inflammation through the activation of several membrane proteins, including the high-affinity receptor for the nerve growth factor (NGF) TrkA and the low-affinity receptor p75^{NTR} (Bulbarelli et al., 2009; Matrone et al., 2009, 2011). The Aβ precursor protein (APP) and presenilin 1 (PS1) are known to be critically involved in Aβ regulation and build up; in particular, PS1, as a part of the gamma-secretase intramembrane protease complex, causes the cleavage of APP at several points within a small region of the protein, and this results in the production of amyloid peptide of various lengths (Golde and Eckman, 2003). In addition, Hoe et al. (2009) suggested a relevant role of APP in synaptic activity, and the synaptic loss is known to be strictly associated with age-related neurocognitive impairments (Terry et al., 1991).

Among the proteins that are found exclusively in neuronal presynaptic terminals, synapsin Ia (Syn-Ia), which is known to play a key role in neurotransmission and synaptic plasticity, is widely recognized as a reliable marker of synaptogenesis and synaptic remodeling (Birch et al., 2013; Cesca et al., 2010).

Aging retardation seems to be strictly dependent on the action of the sirtuin family, which are NAD$^+$-dependent deacetylases whose overexpression has been shown to increase lifespan. Seven sirtuins (SIRT1-7) have been identified in humans and, among these, SIRT3 has been found to be exclusively mitochondrial (Finkel et al., 2009). Since neurons are particularly prone to energy depletion and oxidative stress (Du et al., 2003), SIRT3 has recently emerged as a potential neuroprotective determinant. Indeed, some authors have suggested that SIRT3 could promote healthy aging mainly by regulating the mitochondrial redox status, thus counteracting the age-related oxidative damage (Someya et al., 2010).

We previously demonstrated the involvement of the BDNF-related pathway in the cerebral response of adult mice undergoing a long-term exercise protocol (Falone et al., 2012a). However, BDNF is known to induce neuronal apoptosis when acting as a pro-neurotrophin (proBDNF) under pathological conditions and aging, through the activation of p75^{NTR} and the co-receptor sortilin (Perovic et al., 2013; Volosin et al., 2006).

In view of the crucial roles that the parameters described above may play in maintaining neuronal homeostasis and in preserving cerebral function during aging, in this work we investigated the possible effects of middle age-initiated exercise on the following end-points within the CD-1 mice brain cortex: a) amyloidogenic pathway, b) NGF/proBDNF signaling; and c) Syn-Ia and SIRT3 protein expression. The experimental design involved non spontaneous exercise since some preeminent authors have argued that forced exercise protocols are more similar to human exercise practice and less likely dependent on genetic differences between good and poor runners (Radak et al., 2006).

Our findings have shown that four-month running is able to counteract the main age-related impairing effects on the parameters considered within the mouse brain cortex, although such beneficial actions exhibit a peculiar time-dependent pattern.

2. Materials and methods

2.1. Chemicals and antibodies

Rabbit anti-NGF (cat. sc-548), monoclonal anti-proBDNF (cat. sc-65513), rabbit anti-pSyn-Ia (cat. sc-135708) and rabbit anti-SIRT3 (cat. sc-99143) antibodies were supplied by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Rabbit anti-TrkA phospho Y683 + Y684 (cat. ab79766), anti-p75^{NTR} (cat. ab8875) and anti-β-actin (cat. ab8227) antibodies were purchased from Abcam (Cambridge, UK). The anti-rabbit peroxidase-conjugated secondary antibody (cat. PI-1000) was provided by Vector Laboratories, Inc. (Burlingame, CA, USA). Sigma Aldrich (St. Louis, MO, USA) supplied the anti-mouse peroxidase-conjugated secondary antibody (cat. A9044), the anti-amyloid precursor protein (C-terminal) (cat. A8717), the rabbit anti-presenilin 1 (cat. P7854) antibodies and all the chemicals, substrates and reagents which are not listed elsewhere. Acrylamide/bis acrylamide (cat. 1610125), premixed Laemmli sample buffer (cat. 1610737), Kaleidoscope prestained standards (cat. 1610324) and Immuno-Blot PVDF Sandwiches (cat. 1620239) were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Thermo Fisher Scientific (Rockford, IL, USA) provided the metal-enhanced 3′,3-diaminobenzidine (DAB) substrate kit (cat. 34065) and the bicinchoninic acid (BCA) protein assay kit (cat. 23225). The Human/Rat β-amyloid (42) ELISA kit was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan; cat. 290-62601).

2.2. Animals and running protocol

In this study, 9-mo old CD-1 female mice (45–50 g; N = 48; Harlan Laboratories, Inc., Frederick, MD, USA) were acclimatized for 10 days in the laboratories of the Excellence Research Centre on Aging of the University Foundation "G. d'Annunzio" of Chieti (Italy) to the housing conditions (22 ± 2 °C, 12–12 h light–dark cycle, with lights on from 8 a.m. to 8 p.m., free access to water and food, 6 animals per cage). The familiarization and running protocols used in this research were already used in previous works (Falone et al., 2012a, 2012b). This exercise was recognized as moderate based on the intensity at 65% to 70% of the maximal oxygen uptake (Fernando et al., 1993). Briefly, the animals were familiarized to the Exer 3/6 motorized low-noise treadmill (Columbus Instruments, Columbus, OH, USA) for 2 weeks (9 m/min for 10 min, 5 days/week). Mice were randomly assigned to four groups (12 mice each): mice undergoing a treadmill-based running program for 2 or 4 months (E2 and E4, respectively), and mice undergoing a sedentary regimen for 2 or 4 months (S2 and S4, respectively) (Fig. 1). Exercised groups underwent the training program (warm-up at 5 m/min for 3 min, running at 13 m/min for 20 min, cool-down at 5 m/min for 3 min, zero inclination, 5 days/week), starting from 10 min/day and reaching the final work load by incrementing 1 min every day. Mice were motivated to run by gentle hand prodding, without electric shocks in order to minimize the stress. Mice were observed while exercising to ensure continuous running and to monitor signs of undue stress. Sedentary groups were exposed to the same environmental conditions (handling, treadmill motor noise, vibration and deprivation of food and water) while exercising subjects were performing the running sessions. Food consumption and animal weight were daily monitored throughout the experiment. 24 h after the last training session, mice were rapidly sacrificed by decapitation and brain cortices were surgically removed and stored at −80 °C until further analyses. Every possible effort was made in order to minimize both the number and the suffering of used animals, according to the principles of the Declaration of Helsinki and to the European Community Council (86/609/CEE).

2.3. Western immunoblotting

Brain cortices were homogenized and sonicated (cycle 0.5—amplitude 50%) in 5 vol of RIPA buffer containing 1% protease inhibitor cocktail and 2% phosphatase inhibitor cocktails I and II. Extracts were centrifuged at 15,000 ×g for 30 min at 4 °C and total protein content was assessed by using the bicinchoninic acid (BCA) method, with bovine serum albumin (BSA) as the standard (Smith et al., 1985). Twenty-five micrograms of denatured proteins from each sample were run on polyacrylamide denaturing gels (12–15%) and bands were transferred onto methanol-activated polyvinylidene fluoride (PVDF) sheets by wet electrophoretic transfer (Laemmli, 1970; Towbin et al., 1979). Non-specific binding sites were blocked with 5% (w/v) BSA in Tris-buffered saline containing 0.05% (v/v) Tween 20 (TBS–T) for 1 h. Membranes were then incubated
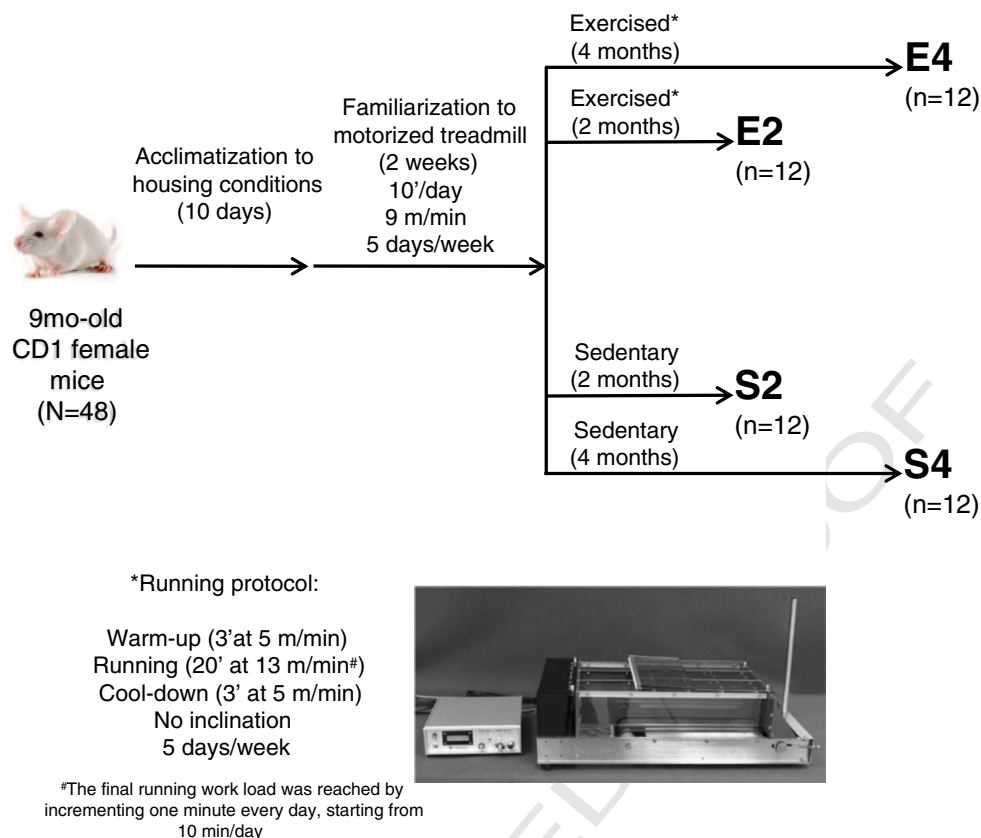
Fig. 1. Experimental design.

201 overnight at 4 °C with TBS-T containing primary antibodies, by using
202 the following dilutions: anti-proBDNF (1:100), anti-NGF (1:200), anti-
203 pTrkA (1:400), anti-PS1 (1:10,000), anti-APP (1:4000), anti-pSyn-Ia
204 (1:100), anti-β-actin (1:1000), anti-p75^{NTR} (1:1000), and anti-SIRT3
205 (1:150). PVDF sheets were washed 3 times with TBS-T (5 min each)
206 and incubated with TBS-T containing the appropriate horseradish
207 peroxidase-conjugated secondary antibody (dil. 1:2000) for 2 h. Mem-
208 branes were washed 3 times with TBS (5 min each) and the immune
209 complexes were detected by using metal-enhanced DAB substrate kit,
210 as suggested by the supplier. Bands were digitally acquired and proc-
211 essed by using the Nonlinear Dynamics TotalLab software. Results
212 were normalized to the signals corresponding to the β-actin house-
213 keeping protein, and given as arbitrary units (AU). The experiments
214 were performed in triplicate.

215 *2.4. ELISA-based detection of Aβ*

216   We used a mAb-based ELISA kit for the quantitative determination
217 of Aβ in cortex homogenates (Scheuner et al., 1996). Briefly, samples
218 were homogenated in 70% formic acid and centrifuged at 100,000 ×g
219 for 1 h at 4 °C. Supernatants were neutralized with 19 volumes of 1 M
220 Tris-base. 100 μL of samples or standard (pure β-amyloid-42, range
221 0–50 pmol/L) were loaded in quadruplicate into a 96-well microplate
222 and incubated at 4 °C overnight. The microplate was washed three
223 times by using the Wash solution 1× provided by the kit. Wells were in-
224 cubated 1 h at 4 °C, in the presence of 100 μL of HRP-conjugated Ab and,
225 subsequently, washed three times with Wash solution 1, as suggested
226 by the manufacturer. Wells were incubated with 100 μL of TMB sub-
227 strate (included in the kit) for 30 min at room temperature in the
228 dark. The Stop solution was added and the microplate was kept for
229 20 s on an orbital shaker (50 rpm), in order to ensure adequate mixing.
230 The plate was read at 450 nm in a Victor3 ELISA reader (Applied

231 Biosystems — Life Technologies, Carlsbad, CA, USA) and all the calcula-
232 tions were carried out as suggested by the manufacturer.

233 *2.5. Statistical analysis*

234   Statistical analyses were performed by using Statsoft Statistica 7 and
235 SyStat SigmaStat v3.5. Results were given as means ± standard devia-
236 tions. All dependent variables were processed by two-way ANOVA anal-
237 ysis. Post-hoc Newman–Keuls tests were used when appropriate. The
238 null hypothesis was rejected with P < 0.05.

3. Results

239

240 *3.1. NGF and Aβ pathway: effects of age and long-term exercise*

241   The transition from 12 to 14 months of age induced a significant de-
242 crease in NGF expression (Fig. 2A, P < 0.01, S4 vs S2) and a marked in-
243 crease in TrkA activation (Fig. 2B, P < 0.01, S4 vs S2). Four-month
244 running did not affect NGF expression, with respect to age-matched
245 mice (Fig. 2A, E4 vs S4). However, the four-month running totally
246 reverted the age-dependent increase in pTrkA expression (Fig. 2B,
247 P < 0.01, E4 vs S4), while two-month running strongly decreased
248 NGF levels (Fig. 2A, P < 0.001, E2 vs S2) but did not affect significant-
249 ly pTrkA amounts (Fig. 2B).
250   A significant increase in the APP protein level occurred after the
251 transition from 12 to 14 months of age (Fig. 3A, P < 0.01, S4 vs S2),
252 whereas no age-induced change in PS1 expression was detected
253 (Fig. 3B, S4 vs S2). Aβ level increased significantly with aging (Fig. 3C,
254 P < 0.001, S4 vs S2).
255   Interestingly, PS1 protein level was significantly reduced after four-
256 month regular running, with respect to age-matched animals (Fig. 3B,
257 P < 0.001, E4 vs S4), while no effect of four-month exercise was found
258 on APP level (Fig. 3A, E4 vs S4). Coherently, the four-month exercise
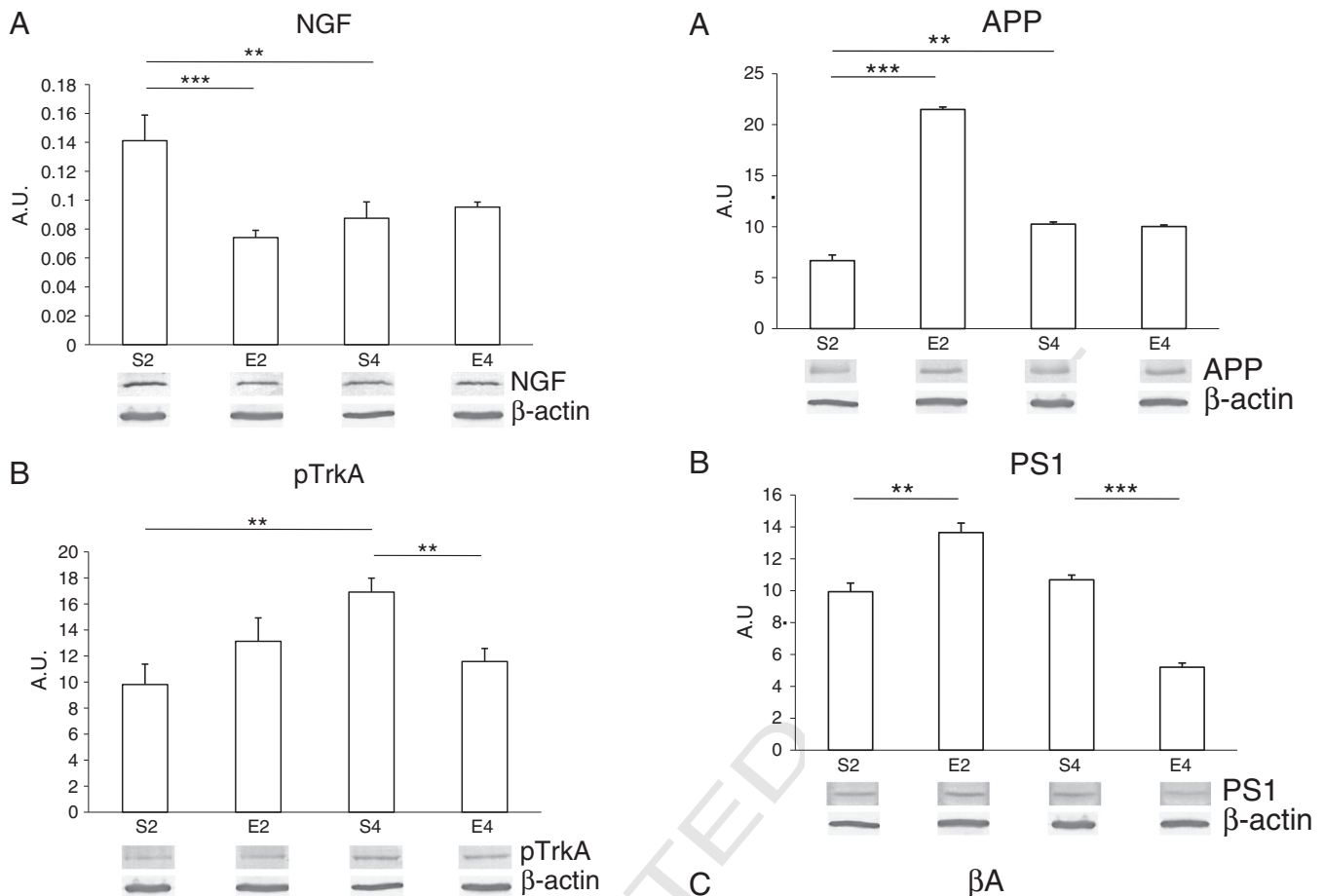
Fig. 2. NGF-dependent signaling in brain cortices of mice after a long-term moderate treadmill running. Western immunoblot against the nerve growth factor (NGF) (panel A) and its phosphorylated high affinity tyrosinekinase B receptor (pTrkB) (panel B) in brain cortices of middle-aged CD1 female mice after two- or four-month moderate and regular treadmill-based exercise program (E2 or E4, respectively); age-matched sedentary animals (S2, S4) were used as controls (n = 6 per group). Immunosignals were normalized against the housekeeping protein β-actin. Representative Western immunoblots of four independent experiments were reported. Values were given as means ± std. dev. and the level of statistical significance was computed by using two-way ANOVA and post-hoc Newman–Keuls test: *P < 0.05; **P < 0.01; ***P < 0.001.

259 regimen significantly reduced the age-related increase of Aβ (Fig. 3C,
260 P < 0.001, E4 vs S4). On the contrary, the two-month running pro-
261 tocol caused a conspicuous increase in both APP (P < 0.001) and
262 PS1 (P < 0.01) proteins, in comparison with the age-matched sedentary
263 mice (Fig. 3A and B) (E2 vs S2). However, no change in Aβ level was
264 found after two-month running, with respect to age-matched sedentary
265 rodents (Fig. 3C, E2 vs S2).

266 *3.2. SIRT3 expression: effects of age and long-term exercise*

267   We found that SIRT3 protein expression declined markedly with age
268 (P < 0.001, S4 vs S2) (Fig. 4). The four-month running protocol inhibited
269 the age-related down-regulation of SIRT3 (P < 0.001, E4 vs S4), whereas
270 an opposite effect was observed in two-month exercised mice, which
271 showed a significant decrease of SIRT3 protein level, with respect to
272 age-matched non-exercised rodents (P < 0.001, E2 vs S2) (Fig. 4).

273 *3.3. proBDNF pathway: effects of age and long-term exercise*

274   The protein level of sortilin was markedly increased after the transi-
275 tion from 12 to 14 months of age (Fig. 5A, P < 0.01, S4 vs S2), while
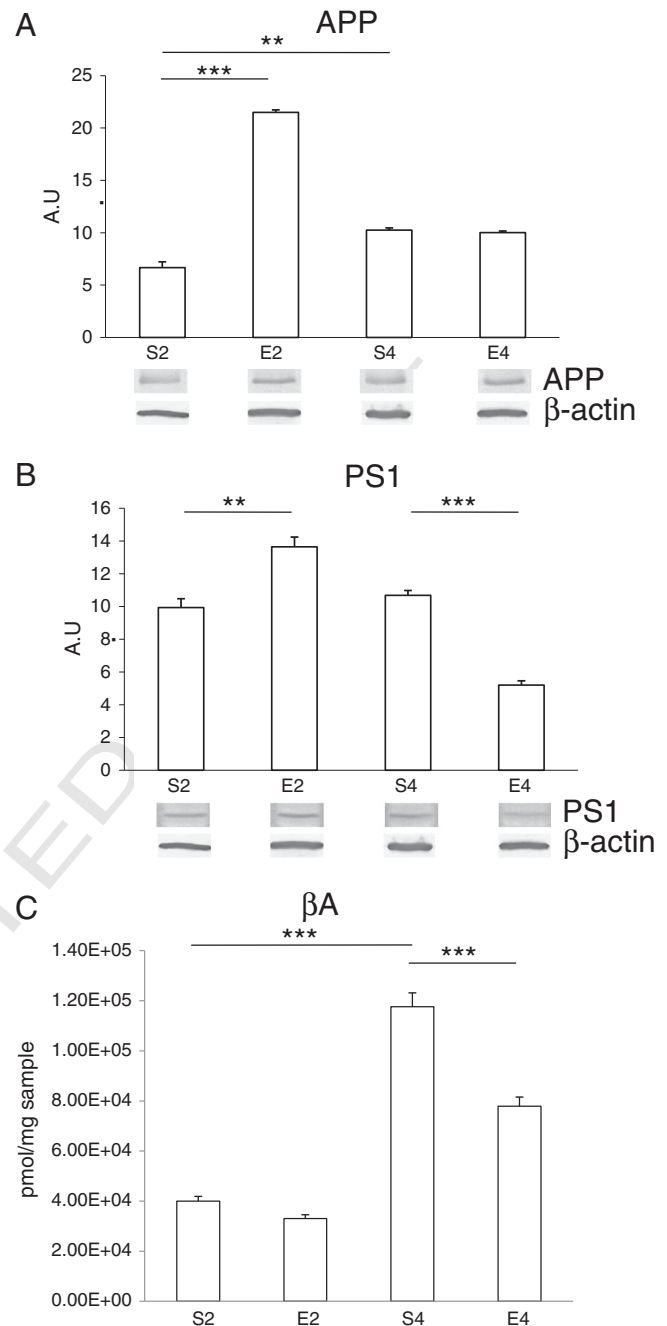
Fig. 3. Amyloidogenic pathway in brain cortices of mice after a long-term moderate treadmill running. Western immunoblot against the beta amyloid precursor (APP) (panel A) and presenilin 1 (PS1) (panel B), and ELISA-based determination of Aβ1-42 (panel C) in brain cortices of middle-aged CD1 female mice after two- or four-month moderate and regular treadmill-based exercise program (E2 or E4, respectively); age-matched sedentary animals (S2, S4) were used as controls (n = 6 per group). Immunosignals were normalized against the housekeeping protein β-actin and representative Western immunoblots of four independent experiments were reported in panels A and B. Values were given as means ± std. dev. and the level of statistical significance was computed by using two-way ANOVA and post-hoc Newman–Keuls test: *P < 0.05; **P < 0.01; ***P < 0.001.

p75[NTR] and proBDNF expressions were not significantly affected by 276
age (Fig. 5B and C) (S4 vs S2).   277
  The levels of both sortilin and p75[NTR] decreased after four-month 278
running (Fig. 5A and B) (P < 0.05 for both proteins, E4 vs S4), whereas 279
proBDNF level showed a decreasing trend even if not statistically signif- 280
icant (Fig. 5C, E4 vs S4), with respect to age-matched sedentary rodents. 281
Interestingly, the two-month running program significantly increased 282
both sortilin (P < 0.01) and proBDNF (P < 0.001) protein expression 283
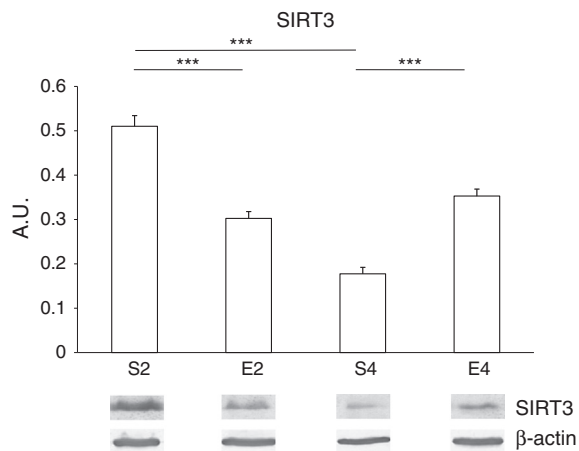
Fig. 4. SIRT3-dependent neuroprotection in brain cortices of mice after a long-term moderate treadmill running. Western immunoblot against the mitochondrial sirtuin 3 (SIRT3) in brain cortices of middle-aged CD1 female mice after two- or four-month moderate and regular treadmill-based exercise program (E2 or E4, respectively); age-matched sedentary animals (S2, S4) were used as controls (n = 6 per group). Immunosignals were normalized against the housekeeping protein β-actin. Representative Western immunoblots of four independent experiments were reported. Values were given as means ± std. dev. and the level of statistical significance was computed by using two-way ANOVA and post-hoc Newman–Keuls test: *P < 0.05; **P < 0.01; ***P < 0.001.

(Fig. 5A and C, E2 vs S2), while the two-month running protocol did not affect significantly the p75^{NTR} level (Fig. 5B, E2 vs S2).

3.4. pSyn-Ia expression: effects of age and long-term exercise

A significant age-dependent reduction of the phospho-synapsin-Ia was detected (Fig. 6) (P < 0.001, S4 vs S2). The four-month exercise regimen totally reverted the age-related decrease of pSyn-Ia protein expression (P < 0.001, E4 vs S4), whereas two-month exercised mice exhibited unchanged levels of pSyn-Ia, with respect to age-matched sedentary subjects (Fig. 6) (E2 vs S2).

4. Discussion

Age-related brain dysfunctions and cognitive impairments are among the most emerging concerns in Western Countries. Lifestyle-based interventions are known to promote successful aging and preserve homeostasis in the CNS (Arslan-Ergul et al., 2013; Camiletti-Moirón et al., 2013). We previously demonstrated that a long-term and moderate treadmill running regimen, even when initiated at the onset of the middle age, positively affected key antioxidant and detoxification enzymatic systems, and improved both molecular damage profiles and BDNF expression within mice brains (Falone et al., 2012a, 2012b). In this study, we explored the possible exercise-induced changes in the expression of proteins mostly involved in synaptic plasticity and in cognitive activity. Unlike what we found for BDNF (Falone et al., 2012a), the long-term exercise failed to rescue the age-related decrease of NGF (Fig. 2A), and the NGF-dependent signaling pathway showed a peculiar profile in sedentary mice, with an up-regulation of the phosphorylated receptor (pTrkA), despite the age-related reduction of the level of its specific ligand NGF (Fig. 2A and B). Interestingly, in non-exercised mice we also found a very significant age-dependent increase in Aβ protein (Fig. 3C). This change was accompanied by a parallel elevation of APP level (Fig. 3A), but not by a significant modification of PS1 protein expression (Fig. 3B). APP and PS1 may form a complex in vivo, and PS1 is necessary for the production of Aβ from APP (Xia et al., 1997). Therefore, it could be argued that APP availability might be the limiting factor in Aβ production and that its increase could be fundamental for the age-dependent Aβ elevation that we observed. In this context, it is important to note that the interruption of NGF signaling in neurons is linked to the activation of the
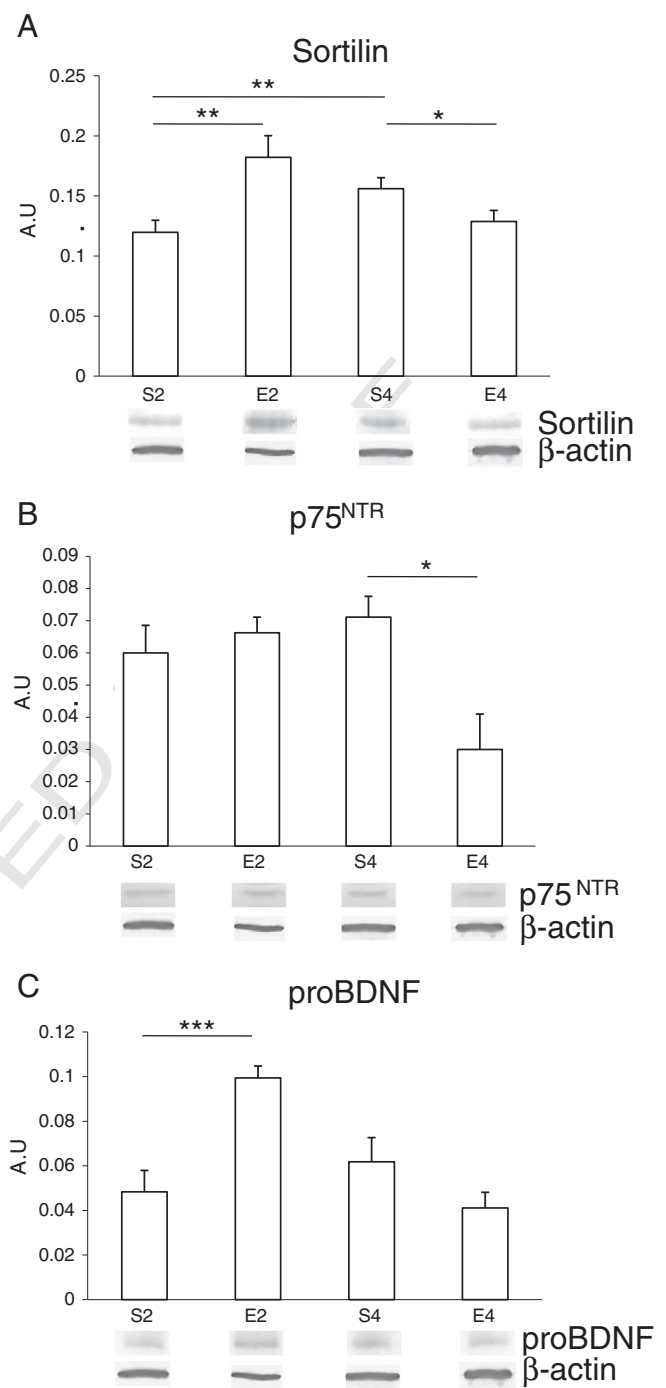
Fig. 5. proBDNF-dependent signaling in brain cortices of mice after a long-term moderate treadmill running. Western immunoblot against the sortilin co-receptor (panel A), p75NTR (panel B) and proBDNF (panel C) in brain cortices of middle-aged CD1 female mice after two- or four-month moderate and regular treadmill-based exercise program (E2 or E4, respectively); age-matched sedentary animals (S2, S4) were used as controls (n = 6 per group). Immunosignals were normalized against the housekeeping protein β-actin. Representative Western immunoblots of four independent experiments were reported. Values were given as means ± std. dev. and the level of statistical significance was computed by using two-way ANOVA and post-hoc Newman–Keuls test: *P < 0.05; **P < 0.01; ***P < 0.001.

amyloidogenic pathway (Matrone et al., 2009). According to the same researchers, NGF deprivation is also linked to NGF-independent phosphorylation of TrkA, which results much higher than that observed in the presence of NGF. On this basis, it could be speculated that the deregulation of NGF/TrkA signaling we observed in sedentary mice might be involved in the amyloidogenic pathway, thus promoting Aβ formation. Interestingly, the four-month exercise program induced a surprising

pSyn-Ia
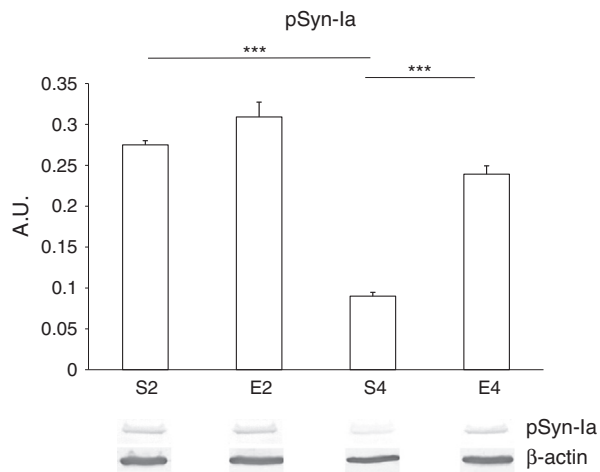
Fig. 6. phospho-synapsin-Ia in brain cortices of mice after a long-term moderate treadmill running. Western immunoblot against the phospho-synapsin-Ia in brain cortices of middle-aged CD1 female mice after two- or four-month moderate and regular treadmill-based exercise program (E2 or E4, respectively); age-matched sedentary animals (S2, S4) were used as controls (n = 6 per group). Immunosignals were normalized against the house-keeping protein β-actin. Representative Western immunoblots of four independent experiments were reported. Values were given as means ± std. dev. and the level of statistical significance was computed by using two-way ANOVA and post-hoc Newman–Keuls test: *P < 0.05; **P < 0.01; ***P < 0.001.

and strong reduction of Aβ levels (Fig. 3C), when compared to the age-matched sedentary controls. Furthermore, PS1 expression resulted significantly decreased in four-month-exercised mice (Fig. 3B), whereas APP level remained unchanged (Fig. 3A). Therefore, the beneficial effect of long-term exercise on cortical Aβ production could be related to a strong depressing effect on the activity of the molecular complex responsible for the gamma-secretase-mediated cleavage of APP. However, it cannot be ruled out that the strong inhibiting effect induced by 4-mo running on the age-related TrkA over-activation (Fig. 2A and B) could also contribute to the decrease in Aβ production that we observed. In fact, as already discussed, some authors have found a link between NGF-independent TrkA phosphorylation and increased amyloidogenesis (Matrone et al., 2009). It is worthy of note that the exercise-dependent reduction of Aβ levels we detected in this study could partially explain the improvement of oxidative damage profile in the cortex of mice undergoing the same exercise protocol that we previously reported (Falone et al., 2012a), which was in line with other studies (Cechetti et al., 2008, 2012; Leite et al., 2012). Indeed, it is well known that the major detrimental cellular effects of Aβ are crucially mediated by ROS over-production (Khandelwal et al., 2011; Yan et al., 2013). To our knowledge, this is the first report describing that a late-life enforced program of long-term moderate exercise affects Aβ-related molecular endpoints within the mouse brain cortex. We strongly believe that these findings may deserve further attention due to their potential relevance in the prevention of age-related neurodegenerations.

It is broadly accepted that precursors and mature forms of neurotrophins (NTs) have distinct and often opposing activities, and the efficiency of neurotrophic pathways is also highly dependent on the ratio of pro- to mature peptides and on the presence of p75^NTR and co-receptor sortilin (Friedman, 2010 Sun et al., 2012). Moreover, precursors and mature forms may differentially affect cognitive functions. In support of this, proBDNF has been shown to evoke long-term depression and synaptic retraction in the hippocampus, whereas mature BDNF has been demonstrated to mediate synaptic strengthening and consolidation by the induction of long-term potentiation (LTP) (Egashira et al., 2010). Therefore, an imbalance between these two forms and the processes underlying the release or the enzymatic conversion of pro- to mature NT forms are considered critical factors for the onset and progression of some neurological disorders. In this

context, the pivotal role of sortilin co-receptor should also be taken into account (Nykjaer and Willnow, 2012; Skeldal et al., 2012).

By comparing S2 and S4 brain cortices, we found a significant difference in the expression of sortilin, whereas the age progression did not change significantly p75^NTR or proBDNF protein levels (Fig. 5A, B and C, respectively). As already reported, our study evidenced an age-dependent increase in the activity of the amyloidogenic pathway, and this can explain, at least in part, the elevation of sortilin. Indeed, according to Saadipour et al. (2013), human AD brains and brains of 6-mo old APPswe/PS1dE9 transgenic mice exhibit higher sortilin expression levels than those detected in age-matched normal brains of humans and wild-type mice. In addition, as established by the same authors, SH-SY5Y cells treated with different concentrations of Aβ42 oligomers showed a strong up-regulation of sortilin (Saadipour et al., 2013). For the first time, we observed that the four-month enforced and moderate running program induced a significant decrease of sortilin protein level (Fig. 5A). Given the already discussed link between sortilin and amyloidogenesis, this could be partially due to the exercise-induced reduction in Aβ42 levels. The sortilin-p75^NTR complex is known to mediate cell death processes through the binding to the pro-domain of proneurotrophins (Jansen et al., 2007), and the molecular complex between sortilin and p75^NTR has been established to regulate synapse elimination and axonal retraction (Singh et al., 2008). It is worthy of note that several age-dependent cognitive declines have been reported during the transition through the biological time period we investigated here (Fouquet et al., 2011), therefore this age range appeared as an adequate time window to study exercise-dependent effects within the mouse CNS. Indeed, as detected for other markers of cerebral function, our results revealed an imbalance in proBDNF-sortilin-p75^NTR-related intracellular signaling occurring as the animals aged in absence of exercise. For the first time to our knowledge, our results suggested that a middle age-initiated regular running regimen may still be able to counteract the molecular processes responsible for the formation of the complex p75NTR-sortilin-proBDNF and the possible consequent age-related cognitive impairment.

Synapsin is an effector in synaptogenesis and plasticity (Vara et al., 2009). Other authors established that at 12–14 months of age, a normal expression of pSyn-I was crucial for preserving mouse object-recognition memory from age-dependent cognitive decline (Corradi et al., 2008). In our study, a significant age-dependent decrease in synapsin Ia phosphorylation was observed within the brain cortex of sedentary mice (Fig. 6), thus supporting data provided by other authors who found a strong age-dependent decrease of synapsin I protein levels in rodents (Sharma et al., 2010). Again, this age range appeared as a very useful time period to study the possible exercise-dependent effects within a mouse CNS exhibiting early signs of age-related impairments. Interestingly, the four-month running protocol partially inhibited such an age-dependent reduction of pSyn-Ia (Fig. 6), thus suggesting that an enforced and moderate running regimen, even when initiated in midlife, could still counteract the likely deleterious effects of age on synaptogenesis and plasticity. As appropriate BDNF levels are thought to be needed in order to modulate synapsin I phosphorylation (Jovanovic et al., 1996), our previous findings regarding a significantly reduced BDNF expression within the cortex of middle aged mice (Falone et al., 2012a) are quite coherent with the results we are presenting here.

Sirtuins mediate some anti-aging effects of caloric restriction, fasting and exercise in several organs (Kincaid and Bossy-Wetzel, 2013). Particular interest is raising around SIRT3, as mitochondrial sirtuins are thought to play key roles in preserving cells from major age-related impairments in oxidative and energy dysfunctions (Duan, 2013). SIRT3 over-expression has been shown to increase neuronal lifespan (Weir et al., 2012) and to protect neurons against excitotoxic injury (Kim et al., 2011). These effects seem to be due to the SIRT3-mediated improvement of the mitochondrial function and to the reduction of ROS-dependent oxidative damage (Tseng et al., 2013). In this study, we have shown a strong age-dependent decrease in SIRT3 expression

within the mouse brain cortex (Fig. 4). However, the late-onset running program was able to prevent the age-related reduction in SIRT3 protein level (Fig. 4). So far, few researchers have investigated the effects of aging and exercise on SIRT3 expression. Lanza et al. (2008) demonstrated that regular cycling or running prevented the age-related reduction of SIRT3 protein expression in human muscles. Conversely, Koltai et al. (2011) found no exercise-dependent effect on SIRT3 protein levels within the rodent CNS. These results are not in accordance with ours and this could be due to the different animal models, ages and exercise protocols used. In particular, Koltai et al. (2011) studied hippocampal formations of very old Wistar rats (26 months) and after a long-term running protocol. Therefore, the lack of exercise-dependent responses on SIRT3 expression in Koltai and co-workers' study could be due to the fact that the hippocampal milieu of 26-mo old rats could be too compromised to respond adaptively to a chronic and severe stressor (6-wk running regimen). In fact, the progressive dysfunction of regulatory mechanisms is thought to be crucially linked to the aged phenotype, and this could reduce the responsivity of the major homeostatic systems (Ferrucci et al., 2010).

To the best of our knowledge, our results indicated for the first time an age-dependent reduction of SIRT3 levels in brain cortices of middle aged mice. In addition, we have also demonstrated for the first time that a four-month moderate running regimen can still revert the age-related decline in SIRT3 levels, even when the exercise protocol is initiated lately in life. Taking into account the SIRT3-dependent enhancement of antioxidant defenses, this result is in strong accordance with our previous observation of an improved redox and antioxidant status within mouse brain cortices following a long-term running regimen (Falone et al., 2012a).

Taken together, our findings suggest that a four-month moderate exercise program is able to trigger multiple responses within the mouse brain cortex through the activation of anti-amyloidogenic (Fig. 3), pro-survival (Fig. 5), synaptogenic (Fig. 6) and neuroprotective (Fig. 4) pathways, even when the exercise regimen is initiated in midlife (see the synopsis in Fig. 7).

However, in our study we found that most of the beneficial actions of the exercise regimen appeared only after four months. This could be due to the fact that the regular running protocol was initiated at the onset of the middle age, that is a biological period in which some initial biomolecular and functional impairments are known to occur within mice brains (Aguiar et al., 2011; Fouquet et al., 2011). In particular, our results suggest the details of a possible adaptive response occurring in brain cortices, in which the two APP-related pathways are co-regulated simultaneously. Normally, the majority of the cellular APP pool is processed through the non-amyloidogenic pathway by an α-secretase cleavage resulting in release of soluble APPα (sAPPα) to the extracellular space. In 2-mo exercised mice we observed a conspicuous increase in both APP and PS1 protein expression, as well as a relevant increase in sortilin receptor (Figs. 3A, B and 5A, respectively). In addition, 2-mo exercised mice exhibited low cortical levels of NGF (Fig. 2A). In neurons, sortilin is known to induce the production of sAPPα, which suggests that sortilin promotes α-secretase activity in neuronal systems (Gustafsen et al., 2013). Some researchers have established that NGF-deprived mice exhibit increased APP processing (Araki and Wurtman, 1998; Capsoni et al., 2013; Matrone et al., 2008). In addition, other researchers have identified a peculiar role of NGF deprivation in regulating neurodegeneration-related pathways through APP cleavage and release of tumor necrosis factor (TNF)-like endogenous agonists (Nikolaev et al., 2009). Hence, on one hand, 2-mo running induced a decline in NGF support, thus favoring APP processing towards neurodegenerative events. On the other hand, we think that the sortilin-mediated formation of non-toxic sAPPα tried to counterbalance the amyloidogenic pathway and to prevent the production of toxic Aβ.

Our study also revealed an intriguing biphasic profile in the SIRT3-based response of brain cortex to the running regimen. In particular, 2-mo moderate exercise lowered SIRT3 protein levels in mouse brain cortex (Fig. 4). This finding is very coherent with the observation of an increased oxidative damage resulting from 2-mo exercise in the same animal model (Falone et al., 2012a). In fact, as shown by Tseng et al. (2013), SIRT3 can protect cells by improving the redox function and reducing ROS-dependent oxidative damage. Other authors have clearly established the key role of SIRT3 in protection against oxidative damage, by demonstrating that Sirt3 knockout mice exhibited increased oxidative stress in skeletal muscle (Jing et al., 2011). Therefore, the decline we have observed here in SIRT3-dependent neuroprotection against oxidative stress can be easily associated with the higher protein carbonylation and lipid peroxidation detected in the brain cortices of CD-1 mice after 2-mo treadmill running (Falone et al., 2012a).

Obviously, the transient worsening of the biomolecular neuroprotective milieu we revealed within the brain cortex of 2-mo exercised mice should not be considered as a permanent imbalance. Indeed, our findings highlight the importance of developing and maintaining moderate and regular exercise habits for very long periods, especially when the physical activity program is initiated at the onset of the middle age. In this regard, temporary imbalances in homeostatic systems are known to act as stressors through which a prolonged physical exercise regimen could activate long-lasting systemic and cellular hormetic responses in mammals (Radak et al., 2008b; Ji et al., 2010).

In conclusion, our results suggest that in the mouse brain cortex an enforced, moderate long-term running program initiated in the middle age activates critical pro-survival and neuroprotective pathways, and protects the mouse CNS from age-related impairments in synaptic function and from amyloidogenic events. On the other hand, we have also shown that moderate treadmill running requires a time lag to activate compensatory processes which are aimed at improving the patterns investigated. The lack of cognitive assessments represents a major limitation of this paper. However, the aim of this work was to investigate whether and how mice brain cortex molecular environment could be affected by an enforced, moderate and regular lately-initiated running regimen. Since we consider important to know if a moderate running program of 2 and 4 months may reverse aging-dependent changes in cognition-related behaviors, we are planning to investigate memory and learning capacities in mice undergoing the same exercise regimen that we have used in this study. Although we are aware of the limits of our molecular investigation, we believe that our findings strongly suggest that a four-month running protocol was still able to counteract many biomolecular impairments occurring within the mouse brain cortex during the transition from adult to middle age. It should also be noted that the brain aging process in a rodent may involve pathways and processes more complex than those observed in the particular time interval we explored in this study. However, this investigation tried to shed new lights on the physiological responses and molecular targets activated by regular and moderate physical exercise initiated
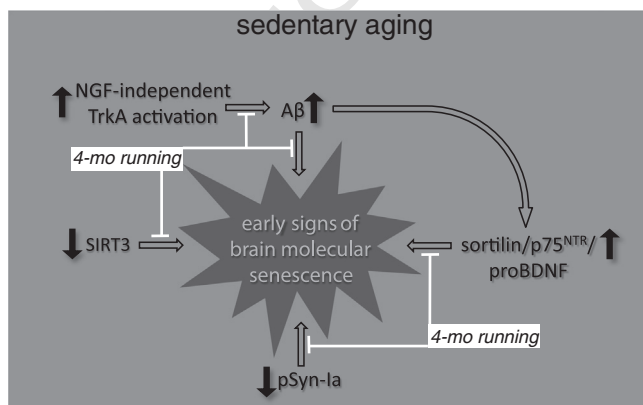
Fig. 7. Synoptic diagram showing the main effects of a lately-initiated 4-mo moderate running program on amyloidogenic, synaptogenic and neuroprotective pathways within the mouse brain cortex.

in the middle age, and strengthens the relevance of developing non-pharmacological interventions aimed at modulating and retarding the brain senescence process.

Conflict of interest

Q3   The authors have no conflicts of interests.

References

Aguiar Jr., A.S., Castro, A.A., Moreira, E.L., Glaser, V., Santos, A.R., Tasca, C.I., Latini, A., Prediger, R.D., 2011. Short bouts of mild-intensity physical exercise improve spatial learning and memory in aging rats: involvement of hippocampal plasticity via AKT, CREB and BDNF signalling. Mech. Ageing Dev. 132 (11–12), 560–567. http://dx.doi.org/10.1016/j.mad.2011.09.005.

Ahlskog, J.E., Geda, Y.E., Graff-Radford, N.R., Petersen, R.C., 2011. Physical exercise as a preventive or disease-modifying treatment of dementia and brain aging. Mayo Clin. Proc. 86 (9), 876–884. http://dx.doi.org/10.4065/mcp.2011.0252.

Andel, R., Crowe, M., Pedersen, N.L., Fratiglioni, L., Johansson, B., Gatz, M., 2008. Physical exercise at midlife and risk of dementia three decades later: a population-based study of Swedish twins. J. Gerontol. A Biol. Sci. Med. Sci. 63 (1), 62–66.

Araki, W., Wurtman, R.J., 1998. Increased expression of amyloid precursor protein and amyloid precursor-like protein 2 during trophic factor withdrawal-induced death of neuronal PC12 cells. Brain Res. Mol. Brain Res. 56 (1–2), 169–177.

Arslan-Ergul, A., Ozdemir, A.T., Adams, M.M., 2013. Aging, neurogenesis, and caloric restriction in different model organisms. Aging Dis. 4 (4), 221–232.

Birch, A.M., McGarry, N.B., Kelly, A.M., 2013. Short-term environmental enrichment, in the absence of exercise, improves memory, and increases NGF concentration, early neuronal survival, and synaptogenesis in the dentate gyrus in a time-dependent manner. Hippocampus 23 (6), 437–450. http://dx.doi.org/10.1002/hipo.22103.

Bulbarelli, A., Lonati, E., Cazzaniga, E., Re, F., Sesana, S., Barisani, D., Sancini, G., Mutoh, T., Masserini, M., 2009. TrkA pathway activation induced by amyloid-beta (Abeta). Mol. Cell. Neurosci. 40 (3), 365–373. http://dx.doi.org/10.1016/j.mcn.2008.12.006.

Camiletti-Moirón, D., Aparicio, V.A., Aranda, P., Radak, Z., 2013. Does exercise reduce brain oxidative stress? A systematic review. Scand. J. Med. Sci. Sports 23 (4), e202–e212. http://dx.doi.org/10.1111/sms.12065.

Capsoni, S., Amato, G., Vignone, D., Criscuolo, C., Nykjaer, A., Cattaneo, A., 2013. Dissecting the role of sortilin receptor signalling in neurodegeneration induced by NGF deprivation. Biochem. Biophys. Res. Commun. 431 (3), 579–585. http://dx.doi.org/10.1016/j.bbrc.2013.01.007.

Cechetti, F., Fochesatto, C., Scopel, D., Nardin, P., Gonçalves, C.A., Netto, C.A., Siqueira, I.R., 2008. Effect of a neuroprotective exercise protocol on oxidative state and BDNF levels in the rat hippocampus. Brain Res. 1188, 182–188.

Cechetti, F., Worm, P.V., Elsner, V.R., Bertoldi, K., Sanches, E., Ben, J., Siqueira, I.R., Netto, C.A., 2012. Forced treadmill exercise prevents oxidative stress and memory deficits following chronic cerebral hypoperfusion in the rat. Neurobiol. Learn. Mem. 97 (1), 90–96. http://dx.doi.org/10.1016/j.nlm.2011.09.008.

Cesca, F., Baldelli, P., Valtorta, F., Benfenati, F., 2010. The synapsins: key actors of synapse function and plasticity. Prog. Neurobiol. 91 (4), 313–348. http://dx.doi.org/10.1016/j.pneurobio.2010.04.006.

Corradi, A., Zanardi, A., Giacomini, C., Onofri, F., Valtorta, F., Zoli, M., Benfenati, F., 2008. Synapsin-I- and synapsin-II-null mice display an increased age-dependent cognitive impairment. J. Cell Sci. 121 (Pt 18), 3042–3051. http://dx.doi.org/10.1242/jcs.035063.

Du, L., Zhang, X., Han, Y.Y., Burke, N.A., Kochanek, P.M., Watkins, S.C., Graham, S.H., Carcillo, J.A., Szabó, C., Clark, R.S., 2003. Intra-mitochondrial poly(ADP-ribosylation) contributes to NAD + depletion and cell death induced by oxidative stress. J. Biol. Chem. 278 (20), 18426–18433.

Duan, W., 2013. Sirtuins: from metabolic regulation to brain aging. Front. Aging Neurosci. 5, 36. http://dx.doi.org/10.3389/fnagi.2013.00036.

Egashira, Y., Tanaka, T., Soni, P., Sakuragi, S., Tominaga-Yoshino, K., Ogura, A., 2010. Involvement of the p75(NTR) signalling pathway in persistent synaptic suppression coupled with synapse elimination following repeated long-term depression induction. J. Neurosci. Res. 88 (16), 3433–3446. http://dx.doi.org/10.1002/jnr.22505.

Falone, S., D'Alessandro, A., Mirabilio, A., Petruccelli, G., Cacchio, M., Di Ilio, C., Di Loreto, S., Amicarelli, F., 2012a. Long term running biphasically improves methylglyoxal-related metabolism, redox homeostasis and neurotrophic support within adult mouse brain cortex. PLoS One 7 (2), e31401. http://dx.doi.org/10.1371/journal.pone.0031401.

Falone, S., D'Alessandro, A., Mirabilio, A., Cacchio, M., Di Ilio, C., Di Loreto, S., Amicarelli, F., 2012b. Late-onset running biphasically improves redox balance, energy- and methylglyoxal-related status, as well as SIRT1 expression in mouse hippocampus. PLoS One 7 (10), e48334. http://dx.doi.org/10.1371/journal.pone.0048334.

Fernando, P., Bonen, A., Hoffman-Goetz, L., 1993. Predicting submaximal oxygen consumption during treadmill running in mice. Can. J. Physiol. Pharmacol. 71 (10–11), 854–857.

Ferreira-Vieira, T.H., Bastos, C.P., Pereira, G.S., Moreira, F.A., Massensini, A.R., 2014. A role for the endocannabinoid system in exercise-induced spatial memory enhancement in mice. Hippocampus 24 (1), 79–88. http://dx.doi.org/10.1002/hipo.22206.

Ferrucci, L., Hesdorffer, C., Bandinelli, S., Simonsick, E.M., 2010. Frailty as a nexus between the biology of aging, environmental conditions and clinical geriatrics. Public Health Rev. 32, 475–488.

Finkel, T., Deng, C.X., Mostoslavsky, R., 2009. Recent progress in the biology and physiology of sirtuins. Nature 460 (7255), 587–591. http://dx.doi.org/10.1038/nature08197.

Fouquet, C., Petit, G.H., Auffret, A., Gaillard, E., Rovira, C., Mariani, J., Rondi-Reig, L., 2011. Early detection of age-related memory deficits in individual mice. Neurobiol. Aging 32 (10), 1881–1895. http://dx.doi.org/10.1016/j.neurobiolaging.2009.11.001.

Friedman, W.J., 2010. Proneurotrophins, seizures, and neuronal apoptosis. Neuroscientist 16 (3), 244–252. http://dx.doi.org/10.1177/1073858409349903.

Geda, Y.E., Roberts, R.O., Knopman, D.S., Christianson, T.J., Pankratz, V.S., Ivnik, R.J., Boeve, B.F., Tangalos, E.G., Petersen, R.C., Rocca, W.A., 2010. Physical exercise, aging, and mild cognitive impairment: a population-based study. Arch. Neurol. 67 (1), 80–86. http://dx.doi.org/10.1001/archneurol.2009.297.

Golde, T.E., Eckman, C.B., 2003. Physiologic and pathologic events mediated by intramembranous and juxtamembranous proteolysis. Sci. STKE 2003 (172), RE4.

Gomez-Pinilla, F., Hillman, C., 2013. The influence of exercise on cognitive abilities. Compr. Physiol. 3 (1), 403–428. http://dx.doi.org/10.1002/cphy.c110063.

Gustafsen, C., Glerup, S., Pallesen, L.T., Olsen, D., Andersen, O.M., Nykjær, A., Madsen, P., Petersen, C.M., 2013. Sortilin and SorLA display distinct roles in processing and trafficking of amyloid precursor protein. J. Neurosci. 33 (1), 64–71. http://dx.doi.org/10.1523/JNEUROSCI.2371-12.2013.

Hoe, H.S., Fu, Z., Makarova, A., Lee, J.Y., Lu, C., Feng, L., Pajoohesh-Ganji, A., Matsuoka, Y., Hyman, B.T., Ehlers, M.D., Vicini, S., Pak, D.T., Rebeck, G.W., 2009. The effects of amyloid precursor protein on postsynaptic composition and activity. J. Biol. Chem. 284 (13), 8495–8506. http://dx.doi.org/10.1074/jbc.M900141200.

Jansen, P., Giehl, K., Nyengaard, J.R., Teng, K., Lioubinski, O., Sjoegaard, S.S., Breiderhoff, T., Gotthardt, M., Lin, F., Eilers, A., Petersen, C.M., Lewin, G.R., Hempstead, B.L., Willnow, T.E., Nykjaer, A., 2007. Roles for the pro-neurotrophin receptor sortilin in neuronal development, aging and brain injury. Nat. Neurosci. 10 (11), 1449–1457.

Ji, L.L., Dickman, J.R., Kang, C., Koenig, R., 2010. Exercise-induced hormesis may help healthy aging. Dose Response 8 (1), 73–79.

Jing, E., Emanuelli, B., Hirschey, M.D., Boucher, J., Lee, K.Y., Lombard, D., Verdin, E.M., Kahn, C.R., 2011. Sirtuin-3 (Sirt3) regulates skeletal muscle metabolism and insulin signalling via altered mitochondrial oxidation and reactive oxygen species production. Proc. Natl. Acad. Sci. U. S. A. 108 (35), 14608–14613. http://dx.doi.org/10.1073/pnas.1111308108.

Jovanovic, J.N., Benfenati, F., Siow, Y.L., Sihra, T.S., Sanghera, J.S., Pelech, S.L., Greengard, P., Czernik, A.J., 1996. Neurotrophins stimulate phosphorylation of synapsin I by MAP kinase and regulate synapsin I-actin interactions. Proc. Natl. Acad. Sci. U. S. A. 93 (8), 3679–3683.

Khandelwal, P.J., Herman, A.M., Hoe, H.S., Rebeck, G.W., Moussa, C.E., 2011. Parkin mediates beclin-dependent autophagic clearance of defective mitochondria and ubiquitinated Abeta in AD models. Hum. Mol. Genet. 20 (11), 2091–2102. http://dx.doi.org/10.1093/hmg/ddr091.

Kim, S.H., Lu, H.F., Alano, C.C., 2011. Neuronal Sirt3 protects against excitotoxic injury in mouse cortical neuron culture. PLoS One 6 (3), e14731. http://dx.doi.org/10.1371/journal.pone.0014731.

Kincaid, B., Bossy-Wetzel, E., 2013. Forever young: SIRT3 a shield against mitochondrial meltdown, aging, and neurodegeneration. Front. Aging Neurosci. 5, 48.

Koltai, E., Zhao, Z., Lacza, Z., Cselenyak, A., Vacz, G., Nyakas, C., Boldogh, I., Ichinoseki-Sekine, N., Radak, Z., 2011. Combined exercise and insulin-like growth factor-1 supplementation induces neurogenesis in old rats, but do not attenuate age-associated DNA damage. Rejuvenation Res. 14 (6), 585–596. http://dx.doi.org/10.1089/rej.2011.1178.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 (5259), 680–685.

Lanza, I.R., Short, D.K., Short, K.R., Raghavakaimal, S., Basu, R., Joyner, M.J., McConnell, J.P., Nair, K.S., 2008. Endurance exercise as a countermeasure for aging. Diabetes 57 (11), 2933–2942. http://dx.doi.org/10.2337/db08-0349.

Leite, H.R., Mourão, F.A., Drumond, L.E., Ferreira-Vieira, T.H., Bernardes, D., Silva, J.F., Lemos, V.S., Moraes, M.F., Pereira, G.S., Carvalho-Tavares, J., Massensini, A.R., 2012. Swim training attenuates oxidative damage and promotes neuroprotection in cerebral cortical slices submitted to oxygen glucose deprivation. J. Neurochem. 123 (2), 317–324. http://dx.doi.org/10.1111/j.1471-4159.2012.07898.x.

Matrone, C., Di Luzio, A., Meli, G., D'Aguanno, S., Severini, C., Ciotti, M.T., Cattaneo, A., Calissano, P., 2008. Activation of the amyloidogenic route by NGF deprivation induces apoptotic death in PC12 cells. J. Alzheimers Dis. 13 (1), 81–96.

Matrone, C., Marolda, R., Ciafrè, S., Ciotti, M.T., Mercanti, D., Calissano, P., 2009. Tyrosine kinase nerve growth factor receptor switches from prosurvival to proapoptotic activity via Abeta-mediated phosphorylation. Proc. Natl. Acad. Sci. U. S. A. 106 (27), 11358–11363. http://dx.doi.org/10.1073/pnas.0904998106.

Matrone, C., Barbagallo, A.P., La Rosa, L.R., Florenzano, F., Ciotti, M.T., Mercanti, D., Chao, M.V., Calissano, P., D'Adamio, L., 2011. APP is phosphorylated by TrkA and regulates NGF/TrkA signalling. J. Neurosci. 31 (33), 11756–11761. http://dx.doi.org/10.1523/JNEUROSCI.1960-11.2011.

Mattson, M.P., 2012. Energy intake and exercise as determinants of brain health and vulnerability to injury and disease. Cell Metab. 16 (6), 706–722. http://dx.doi.org/10.1016/j.cmet.2012.08.012.

Nikolaev, A., McLaughlin, T., O'Leary, D.D., Tessier-Lavigne, M., 2009. APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. Nature 457 (7232), 981–989. http://dx.doi.org/10.1038/nature07767.

Nykjaer, A., Willnow, T.E., 2012. Sortilin: a receptor to regulate neuronal viability and function. Trends Neurosci. 35 (4), 261–270. http://dx.doi.org/10.1016/j.tins.2012.01.003.

Perovic, M., Tesic, V., Mladenovic Djordjevic, A., Smiljanic, K., Loncarevic-Vasiljkovic, N., Ruzdijic, S., Kanazir, S., 2013. BDNF transcripts, proBDNF and proNGF, in the cortex and hippocampus throughout the life span of the rat. Age (Dordr) 35 (6), 2057–2070. http://dx.doi.org/10.1007/s11357-012-9495-6.

Radak, Z., Toldy, A., Szabo, Z., Siamilis, S., Nyakas, C., Silye, G., Jakus, J., Goto, S., 2006. The effects of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. Neurochem. Int. 49 (4), 387–392.

709 Radak, Z., Chung, H.Y., Goto, S., 2008a. Systemic adaptation to oxidative challenge induced
710   by regular exercise. Free Radic. Biol. Med. 44 (2), 153–159. http://dx.doi.org/10.1016/
711   j.freeradbiomed.2007.01.029.
712 Radak, Z., Chung, H.Y., Koltai, E., Taylor, A.W., Goto, S., 2008b. Exercise, oxidative stress
713   and hormesis. Ageing Res. Rev. 7 (1), 34–42.
714 Saadipour, K., Yang, M., Lim, Y., Georgiou, K., Sun, Y., Keating, D., Liu, J., Wang, Y.R., Gai, W.
715   P., Zhong, J.H., Wang, Y.J., Zhou, X.F., 2013. Amyloid beta$_4$ (Aβ_4) up-regulates the
716   expression of sortilin via the p75(NTR)/RhoA signalling pathway. J. Neurochem.
717   127 (2), 152–162. http://dx.doi.org/10.1111/jnc.12383 (Oct).
718 Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T.D., Hardy, J.,
719   Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Viitanen, M., Peskind, E., Poorkaj,
720   P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D., Younkin, S., 1996. Se-
721   creted amyloid beta-protein similar to that in the senile plaques of Alzheimer's dis-
722   ease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to
723   familial Alzheimer's disease. Nat. Med. 2 (8), 864–870.
724 Sharma, S., Singh, R., Kaur, M., Kaur, G., 2010. Late-onset dietary restriction compensates
725   for age-related increase in oxidative stress and alterations of HSP 70 and synapsin 1
726   protein levels in male Wistar rats. Biogerontology 11 (2), 197–209. http://dx.doi.org/
727   10.1007/s10522-009-9240-4.
728 Singh, K.K., Park, K.J., Hong, E.J., Kramer, B.M., Greenberg, M.E., Kaplan, D.R., Miller, F.D.,
729   2008. Developmental axon pruning mediated by BDNF-p75NTR-dependent axon de-
730   generation. Nat. Neurosci. 11 (6), 649–658. http://dx.doi.org/10.1038/nn.2114.
731 Skeldal, S., Sykes, A.M., Glerup, S., Matusica, D., Palstra, N., Autio, H., Boskovic, Z., Madsen,
732   P., Castrén, E., Nykjaer, A., Coulson, E.J., 2012. Mapping of the interaction site between
733   sortilin and the p75 neurotrophin receptor reveals a regulatory role for the sortilin in-
734   tracellular domain in p75 neurotrophin receptor shedding and apoptosis. J. Biol.
735   Chem. 287 (52), 43798–43809. http://dx.doi.org/10.1074/jbc.M112.374710.
736 Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D.,
737   Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein
738   using bicinchoninic acid. Anal. Biochem. 150 (1), 76–85.
739 Someya, S., Yu, W., Hallows, W.C., Xu, J., Vann, J.M., Leeuwenburgh, C., Tanokura, M., Denu,
740   J.M., Prolla, T.A., 2010. Sirt3 mediates reduction of oxidative damage and prevention

741 of age-related hearing loss under caloric restriction. Cell 143 (5), 802–812. http://dx.
742   doi.org/10.1016/j.cell.2010.10.002.
743 Sun, Y., Lim, Y., Li, F., Liu, S., Lu, J.J., Haberberger, R., Zhong, J.H., Zhou, X.F., 2012. ProBDNF
744   collapses neurite outgrowth of primary neurons by activating RhoA. PLoS One 7 (4),
745   e35883. http://dx.doi.org/10.1371/journal.pone.0035883.
746 Terry, R.D., Masliah, E., Salmon, D.P., Butters, N., DeTeresa, R., Hill, R., Hansen, L.A.,
747   Katzman, R., 1991. Physical basis of cognitive alterations in Alzheimer's disease: syn-
748   apse loss is the major correlate of cognitive impairment. Ann. Neurol. 30 (4),
749   572–580.
750 Towbin, H., Staehelin, T., Gordon, J., 1979. Electrophoretic transfer of proteins from poly-
751   acrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl.
752   Acad. Sci. U. S. A. 76 (9), 4350–4354.
753 Tseng, A.H., Shieh, S.S., Wang, D.L., 2013. SIRT3 deacetylates FOXO3 to protect mitochon-
754   dria against oxidative damage. Free Radic. Biol. Med. 63, 222–234. http://dx.doi.org/
755   10.1016/j.freeradbiomed.2013.05.002.
756 Vara, H., Onofri, F., Benfenati, F., Sassoè-Pognetto, M., Giustetto, M., 2009. ERK activation in
757   axonal varicosities modulates presynaptic plasticity in the CA3 region of the hippo-
758   campus through synapsin I. Proc. Natl. Acad. Sci. U. S. A. 106 (24), 9872–9877.
759   http://dx.doi.org/10.1073/pnas.0900077106.
760 Volosin, M., Song, W., Almeida, R.D., Kaplan, D.R., Hempstead, B.L., Friedman, W.J., 2006.
761   Interaction of survival and death signalling in basal forebrain neurons: roles of
762   neurotrophins and proneurotrophins. J. Neurosci. 26 (29), 7756–7766.
763 Weir, H.J., Murray, T.K., Kehoe, P.G., Love, S., Verdin, E.M., O'Neill, M.J., Lane, J.D., Balthasar,
764   N., 2012. CNS SIRT3 expression is altered by reactive oxygen species and in
765   Alzheimer's disease. PLoS One 7 (11), e48225. http://dx.doi.org/10.1371/journal.
766   pone.0048225.
767 Xia, W., Zhang, J., Perez, R., Koo, E.H., Selkoe, D.J., 1997. Interaction between amyloid pre-
768   cursor protein and presenilins in mammalian cells: implications for the pathogenesis
769   of Alzheimer disease. Proc. Natl. Acad. Sci. U. S. A. 94 (15), 8208–8213.
770 Yan, M.H., Wang, X., Zhu, X., 2013. Mitochondrial defects and oxidative stress in
771   Alzheimer disease and Parkinson disease. Free Radic. Biol. Med. 62, 90–101. http://
772   dx.doi.org/10.1016/j.freeradbiomed.2012.11.014.

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