Published in final edited form as:

J Neural Transm. 2010 December ; 117(12): 1353–1358. doi:10.1007/s00702-010-0483-7.

Molecular correlates of spontaneous activity in non-human primates

Amanda C. Mitchell,

Department of Psychiatry, Vanderbilt University, 8130A MRB III, 465 21st Avenue South, Nashville, 37232, USA

Georgina Aldridge,

Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Shawn Kohler,

Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Greg Stanton,

Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Elinor Sullivan,

Oregon National Primate Research Center, Beaverton, OR, USA

Krassimira Garbett,

Department of Psychiatry, Vanderbilt University, 8130A MRB III, 465 21st Avenue South, Nashville, 37232, USA

Vanderbilt Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN, USA

Gabor Faludi,

Department of Psychiatry, Semmelweis University, Budapest, Hungary

Károly Mirnics,

Department of Psychiatry, Vanderbilt University, 8130A MRB III, 465 21st Avenue South, Nashville, 37232, USA karoly.mirnics@vanderbilt.edu

Vanderbilt Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, TN, USA

Judy L. Cameron, and

Oregon National Primate Research Center, Beaverton, OR, USA

Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

William Greenough

Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Abstract

In our monkey model, cortical ARC and BDNF expressions were strongly correlated with spontaneous physical activity. The expressions of ARC and BDNF were inversely correlated with serum CRP levels, suggesting that CRP could be a putative peripheral marker of brain resiliency.

Keywords

Motor cortex; Gene expression; CRP; Physical activity; ARC; BDNF

Introduction

A growing body of evidence suggests that mild (Yaffe et al. 2001), moderate, and vigorous physical activities (Thacker et al. 2008) are neuroprotective, decreasing the risk of many brain disorders including ischemic stroke (Hu et al. 2000; Lee and Paffenbarger 1998), Alzheimer's disease (Yaffe et al. 2001; Larson et al. 2006), and Parkinson's disease (PD) (Thacker et al. 2008). Numerous studies also associate the modern sedentary lifestyle with increased risks for obesity, cardiovascular diseases, type II diabetes (Marwick et al. 2009), and depression (Lawlor and Hopker 2001; Vance et al. 2005).

At the molecular level, exercise is correlated with increased levels of neurotrophic factors [nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and fibroblast growth factor-2 (FGF-2)], synaptic trafficking genes (syntaxin, synapsin I, and synaptotagmin), and activation of signal transduction pathways (CaMKII δ , ERK1/2, and PKC) (Neeper et al. 1996; Molteni et al. 2002; Tong et al. 2001). In a previous study, we found that both BDNF and activity regulated cytoskeletal-associated protein (ARC) are elevated in mice exposed to enriched environments (Lazarov et al. 2005), suggesting that activation of the ARC–BDNF pathway might be a critical mediator of neuroprotective events. ARC is an immediate early gene induced by neuronal excitation with a role in activity-dependent synaptic modification (Steward and Worley 2002), and active behavior in the hippocampus, neocortex, and striatum (Vazdarjanova et al. 2006).

In contrast, high C-reactive protein (CRP) is associated with increased risk for type II diabetes (Lakka et al. 2005), hypertension, cardiovascular disease, and ischemic stroke (Stewart et al. 2009). It is involved in host defense-related functions, recognizes foreign pathogens and damaged cells, and initiates their elimination by interacting with humoral and cell effector systems in the blood (Stenvinkel 2006). In healthy individuals, the concentration of CRP in serum is low (Yeh 2004). As a result of ischemic infarction, CRP binds to phosphocholine expressed on the surface of dead or dying cells to activate complement during the acute phase response (Volanakis and Kaplan 1971; Stenvinkel 2006), and is increased in the bloodstream in response to tissue injury, infection, and other inflammatory stimuli (Futterman and Lemberg 2002). Furthermore, high CRP enhances ischemic tissue damage in rats (Gill et al. 2004), indicating that it may be an active contributor to pathophysiological events.

Recently, it has been shown that exercise can also lower CRP in the blood plasma (Rhyu et al. 2003). This finding, combined with well-established activity-dependent elevation of ARC/BDNF expression in the brain, leads to the hypothesis that there is a relationship between spontaneous physical activity, CRP plasma levels, and ARC/BDNF expression in the brain. To test this hypothesis, we measured and correlated CRP serum levels and brain expressions of ARC and BDNF in 11 rhesus monkeys with various levels of spontaneous activity.

Methods

Experimental animals

All experiments were reviewed and approved by the Animal Care and Use Committee of the Oregon National Primate Research Center. Eleven adult female ovariectomized rhesus

monkeys (*Macaca mulatta*) were housed in individual cages (32 × 24 × 27 or 32 × 34 × 27 in.) in a temperature-controlled room (24 ± 2°C), with lights on between 0700 and 1900 hours. Two and a half years prior to the initiation of this study, these monkeys were ovariectomized and placed on a diet higher in fat than standard monkey chow (35% of calories from fat) to approximate the conditions experienced by many post-menopausal women in the Western world (Williams et al. 2003). This diet was formulated at the Oregon National Primate Research Center (ONPRC) (Sullivan et al. 2006; Sullivan and Cameron 2010), following a modification of the recipe developed by Clarkson and colleagues to study diet-induced atherosclerosis (Shadoan et al. 2003; Williams et al. 2003). Monkeys were subsequently placed on a diet of Purina high protein monkey chow (no. 5045; Ralston Purina, St. Louis, MO), supplemented with fresh fruits and vegetables for 2 months. During the first month, available calories were reduced to 30% as compared to baseline on the higher fat diet, and in the second month, the calorie intake was reduced to 60% as compared to baseline.

In vivo experimental measures

Throughout the study, food intake was measured at every meal, body weight was measured weekly, and activity was measured continuously using omnidirectional Actical accelerometers (Respironics, Phoenix, AZ), attached to a loose-fitting metal collar, via previously published methods (Sullivan et al. 2006; Sullivan and Cameron 2010). Percent body fat was determined using dual energy X-ray absorptiometry scans (Sullivan et al. 2006; Sullivan and Cameron 2010).

Collection of brain tissue

Monkeys were deeply anesthetized with ketamine/pentobarbital, quickly decapitated, and the entire brain was removed from the skull. The brain was then hemi-sectioned by a midline sagittal cut. The right hemisphere was cut into ~5-mm thick coronal blocks, flash-frozen in isopentane over dry ice, and stored at -80°C until use (Volk et al. 2000).

RNA sample preparation

The motor cortex was identified using anatomical landmarks, and a 2 × 2 mm tissue cube of transcortical gray matter was dissected from the frozen block using surgical tools under RNase-free conditions. The motor cortex brain material was homogenized and total RNA isolated using TRIzol® reagent (Invitrogen, Carlsbad, CA) with RNA quality assessed via analysis on an Agilent 2100 Bioanalyzer. Only samples with an RIN >7.0 were considered for further analysis.

Quantitative real-time PCR

cDNA synthesis was performed using two independent reverse transcription reactions for each sample with High Capacity cDNA Archive Kit® (Applied Biosystems). For each 100 µl reaction, we used 700 ng of the same total RNA used for microarray analysis. Priming was performed with random hexamers. For each sample, amplified product differences were measured with four independent replicates using SYBR Green chemistry-based detection (Mimmack et al. 2004). β -Actin was used as the endogenous reference gene because it has been established as a stable reference gene in the literature (Chen et al. 2001; Arion et al. 2006, 2007). Primer sequences for β -actin were 5' GAT GTG GAT CAG CAA GCA 3' and 5' AGA AAG GCT GTA ACG CAA CTA 3', for BDNF 5' GAC TGG ACG ACC ACA CTC AAG 3' and 5' CAA CCT GCT CCA GGC TAA TC 3', and for ARC 5' CGC CTG GAG AAG AAT CAG AG 3' and 5' CTC AGC CGG ATT TGA GGA C 3'. The efficiency for each primer set was assessed prior to qPCR measurements, and a primer set was considered valid if its efficiency was >90%. The qPCR reactions were carried out on an ABI

Prism 7300 thermal cycler (Applied Biosystems Inc.), quantified using ABI Prism 7300 SDS software (with the auto baseline and auto threshold detection options selected) and statistically analyzed using Pearson correlations.

CRP measurements

CRP was analyzed using serum that drawn immediately prior to termination of the study, after animals had been on a low fat diet for 2 months following a 3-year period on a 35% fat diet. Serum was stored frozen, warmed to make aliquots and then refrozen before use. A quantitative ELISA kit specific for monkey CRP (Alpha Diagnostic international, 96 well) was used to measure serum levels of CRP according to the manufacturer's instructions. Serum aliquots were diluted with the Elisa kit diluent between 1:200 and 1:800 to cover the range of CRP levels in these animals, and run on a single ELISA plate in triplicate. Results shown were based on a 1:400 dilution, as this range best centered the observed levels within the standard curve, but other dilutions did not alter the conclusions drawn. Optical density of the colored reactant was quantified using an Emax Precision Microplate Reader (Molecular Devices) set to 450 nm, measured twice and averaged. A standard curve was generated using known sample concentrations, run on the same ELISA plate in duplicate. Sigma Plot (Systat) was then used to calculate the unknown concentrations of CRP using the standard curve.

Statistical procedures

BDNF, ARC, CRP, and spontaneous activity measurements were correlated using Pearson product-moment correlation coefficient in Microsoft Excel 2007. The significance of the Pearson product-moment correlation coefficient was calculated using $t = r[(1-r^2)/(N-2)]$. p values were obtained from the t statistic (Lowry and Richard 2010). As higher values indicate lower absolute transcript expression, to denote meaningful correlations, in Fig. 1b–e, qPCR expression data are plotted as a $1/\Delta C_t$.

Results

Spontaneous activity levels in the tested monkeys, measured continuously with accelerometers, showed individual variation of more than an order of magnitude, and encompassed a range of 46,717–760,838 counts/day (mean = 398,152 counts/day, SD = 273,011 counts/day) at the beginning of the calorie restriction diet and 46,478–515,105 counts/day (mean = 244,855 counts/day, SD = 161,681 counts/day) at the end of the calorie restriction diet. Activity measurements at the beginning and end of the calorie restriction diet were highly correlated ($r = 0.85$, $p = 0.000379$), suggesting that diet was not a crucial factor in defining the relative level of spontaneous activity, and that relative activity level was a stable intrinsic characteristic of monkeys over the investigated time window. The monkeys displayed serum concentrations of CRP in the range of 4.82–26.28 mg/L (mean concentration = 12.47, SD = 7.30) (Table 1 in Fig. 1a), while brain expression measurement of BDNF and ARC suggested that both genes were abundantly expressed in the motor cortex, albeit with less individual variability than the CRP and spontaneous activity levels.

As we obtained measurements for continuous variables (spontaneous activity, serum CRP, cortical ARC, and cortical BDNF) to test our hypotheses we cross-correlated the overall data using Pearson product-moment correlation coefficient. As expected from our previously reported findings (Lazarov et al. 2005), the expressions of ARC and BDNF were correlated in the motor cortex ($r = 0.51$, $p = 0.05$) (Fig. 1b). Furthermore, ARC and BDNF activities were also significantly correlated with spontaneous activity ($r = 0.60$, $p = 0.02$ and $r = 0.54$, $p = 0.04$, respectively) (Fig. 1c). In contrast, CRP plasma levels were inversely and significantly correlated with spontaneous activity ($r = -0.64$, $p = 0.01$) (Fig. 1d). Finally, the

CRP levels were also strongly and inversely correlated with the motor cortical expressions of ARC and BDNF ($r = -0.81$, $p < 0.001$ and $r = -0.80$, $p < 0.001$, respectively) (Fig. 1e), raising the possibility of a causal co-regulation between these measurements.

Discussion

This study gave rise to three major findings. First, spontaneous activity in the primate is stable over time and although a monkey's activity level decreases with calorie restriction (Sullivan and Cameron 2010), their activity level relative to that of other monkeys remains stable. Second, spontaneous activity is positively correlated with ARC and BDNF expressions in the motor cortex of non-human primates, and negatively correlated with serum CRP levels. Third, serum CRP levels are strongly and negatively correlated with ARC and BDNF expressions in the motor cortex of non-human primates.

Previous studies in mice, rats, and monkeys show the same general localization of BDNF and ARC to layers II, III, IV, and VI in the cortex (Link et al. 1995; Grinevich et al. 2009; Hofer et al. 1990; Phillips et al. 1990; Zhang et al. 2007), and both are predominantly expressed in the principal (projection) neurons in the cortex. Thus, we believe that the CRP-correlated cortical expression changes occur in the same population of the projection neurons. Nevertheless, this hypothesis will have to be confirmed in the follow up co-localization studies in a different experimental cohort, as our fresh-frozen material is less than which is ideally suited for such experiments.

Activity, CRP, BDNF, and ARC co-regulations raise an important and interesting question: how are peripheral changes in CRP levels in the blood related to the gene expression changes in the motor cortex? Based on the previous literature findings, we believe that this relationship could be causal and related to dynamic changes in brain vascularization: (1) CRP can significantly influence gene expression in the vascular endothelium after 24 h (Wang et al. 2005), (2) increased levels of CRP can increase infarct in rats and enhance ischemic tissue damage (Gill et al. 2004), (3) CRP is also increased in the blood plasma in response to tissue injury, infection, and other inflammatory stimuli (Yeh 2004), (4) CRP causes blood-brain barrier disruption involving the formation of ROS by the NAD(P)H-oxidase (Kuhlmann et al. 2009), (5) brain endothelial cells express higher levels of CRP receptors and have increased vulnerability of brain endothelial cells to CRP following stroke (Kuhlmann et al. 2009), (6) MPTP administration increases CRP levels in non-human primates (De Pablos et al. 2009), and (7) high CRP is associated with increased risk for type II diabetes (Lakka et al. 2005), hypertension, cardiovascular disease, and ischemic stroke (Stewart et al. 2009). Furthermore, exercise results in the reduction of weight and is associated with improvements in CRP (Church et al. 2009).

However, we must also acknowledge the possibility that the correlation between peripheral CRP levels and BDNF/ARC is a result of a more complex, coordinated set of pathophysiological events. This cascade might also involve an insulin-like growth factor (IGF) dependent mechanism: peripheral administration of IGF has also been shown to induce BDNF mRNA expression in the hippocampus and cortex (Carro et al. 2000). Furthermore, exercise increases IGF1 levels in the brain, and IGF1 blocking antibodies administered before a neural injury results in decreased neuroprotection (Carro et al. 2001). Thus, one could hypothesize that the activity-induced increase in IGF1 levels leads to upregulation of BDNF/ARC in the hippocampus and cortex.

Finally, our findings and the literature data raise a critical question: do increased peripheral CRP levels increase the chance of an individual to develop neurodegenerative brain diseases? If so, lowering CRP levels in the periphery might be neuroprotective, and this

could be assessed in various in vitro and in vivo model systems. Further studies of the activity-CRP-ARC-BDNF relationships and the health of the brain are warranted by our findings, and could lead to important new strategies for early detection of, as well as novel treatments for neurodegenerative disorders.

Acknowledgments

The authors are grateful to the Division of Animal Resources at ONPRC for the expert care of the monkeys used in this study, and to Diana Takahashi and Lindsay Pranger for technical assistance with in vivo experiments. We are also thankful to Khine Lwin for outstanding technical assistance with sample preparation and handling. This work was supported by Grants from the National Institute of Health (MH067234, MH079299, MH067346, DK55819, HD18185, and RR00163). Salary support to KM was provided by K02 MH070786.

References

- Arion D, Sabatini M, Unger T, Pastor J, Alonso-Nanclares L, Ballesteros-Yanez I, Garcia Sola R, Munoz A, Mirnics K, DeFelipe J. Correlation of transcriptome profile with electrical activity in temporal lobe epilepsy. *Neurobiol Dis.* 2006; 22:374–387. [PubMed: 16480884]
- Arion D, Unger T, Lewis DA, Levitt P, Mirnics K. Molecular evidence for increased expression of genes related to immune and chaperone function in the prefrontal cortex in schizophrenia. *Biol Psychiatry.* 2007; 62:711–721. [PubMed: 17568569]
- Carro E, Nunez A, Busiguina S, Torres-Aleman I. Circulating insulin-like growth factor I mediates effects of exercise on the brain. *J Neurosci.* 2000; 20:2926–2933. [PubMed: 10751445]
- Carro E, Trejo JL, Busiguina S, Torres-Aleman I. Circulating insulin-like growth factor I mediates the protective effects of physical exercise against brain insults of different etiology and anatomy. *J Neurosci.* 2001; 21:5678–5684. [PubMed: 11466439]
- Chen J, Sochivko D, Beck H, Marechal D, Wiestler OD, Becker AJ. Activity-induced expression of common reference genes in individual CNS neurons. *Lab Invest.* 2001; 81:913–916. [PubMed: 11406652]
- Church TS, Earnest CP, Thompson AM, Priest E, Rodarte RQ, Sanders T, Ross R, Blair SN. Exercise without weight loss does not reduce C-reactive protein: the INFLAME study. *Med Sci Sports Exerc.* 2009; 42(4):708–716. [PubMed: 19952828]
- De Pablos V, Barcia C, Martínez S, Gomez A, Ros-Bernal F, Zamarró-Parra J, Soria-Torrecillas JJ, Hernández J, Ceron JJ, Herrero MT. MPTP administration increases plasma levels of acute phase proteins in non-human primates (*Macaca fascicularis*). *Neurosci Lett.* 2009; 463(1):37–39. [PubMed: 19638294]
- Futerman LG, Lemberg L. High-sensitivity C-reactive protein is the most effective prognostic measurement of acute coronary events. *Am J Crit Care.* 2002; 11:482–486. [PubMed: 12233975]
- Gill R, Kemp JA, Sabin C, Pepys MB. Human C-reactive protein increases cerebral infarct size after middle cerebral artery occlusion in adult rats. *J Cereb Blood Flow Metab.* 2004; 24(11):1214–1218. [PubMed: 15545914]
- Grinevich V, Kollek A, Eliava M, Takada N, Takuma H, Fukazwa Y, Shigemoto R, Kuhl D, Waters J, Seeburg PH, Osten P. Fluorescent Arc/Arg3.1 indicator mice: a versatile tool to study brain activity changes in vitro and in vivo. *J Neurosci Methods.* 2009; 184(1):25–36. [PubMed: 19628007]
- Hofer M, Pagliusi SR, Hohn A, Leibrok J, Barde YA. Regional distribution of brain-derived neurotrophic factor in mRNA in the adult mouse brain. *EMBO.* 1990; 9(8):2459–2464.
- Hu FB, Stampfer MJ, Colditz GA, Ascherio A, Rexrode KM, Willett WC, Manson JC. Physical activity and risk of stroke in women. *JAMA.* 2000; 283:2961–2967. [PubMed: 10865274]
- Kuhlmann CR, Librizzi L, Closhen D, Pflanzner T, Lessmann V, Pietrzik CU, de Curtis M, Luhmann HJ. Mechanisms of C-reactive protein-induced blood–brain barrier disruption. *Stroke.* 2009; 40(4):1458–1466. [PubMed: 19246692]
- Lakka TA, Lakka HM, Rankinen T, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C. Effect of exercise training on serum levels of C-reactive protein in healthy individuals: the heritage family study. *Eur Heart J.* 2005; 26:2018–2025. [PubMed: 15987707]

- Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P, Kukull W. Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. *Ann Intern Med*. 2006; 144(2):73–81. [PubMed: 16418406]
- Lawlor A, Hopker SW. The effectiveness of exercise as an intervention in the management of depression: systematic review and meta-regression analysis of randomised controlled trials. *BMJ*. 2001; 322:763–767. [PubMed: 11282860]
- Lazarov O, Robinson J, Tang YP, Hairston IS, Korade-Mirnic Z, Lee VM, Hersch LB, Sapolsky RM, Mirnic K, Sisodia SS. Environmental enrichment reduces A β levels and amyloid deposition in transgenic mice. *Cell*. 2005; 120:701–713. [PubMed: 15766532]
- Lee IM, Paffenbarger RS. Physical activity and stroke incidence. The harvard alumni health study. *Stroke*. 1998; 29:2049–2054. [PubMed: 9756580]
- Link W, Konietzko U, Kauselmann G, Krugt M, Schwanke B, Freys U, Kuhl D. Somatodendritic expression of an immediate early gene is regulated by synaptic activity. *Proc Natl Acad Sci USA*. 1995; 92:5734–5738. [PubMed: 7777577]
- Lowry, Richard. The significance of a correlation coefficient.. Concepts and applications of inferential statistics. 2010. <http://faculty.vassar.edu/lowry/ch4apx.html>
- Marwick TH, Hordern MD, Miller T, Chyun DA, Bertoni AG, Blumenthal RS, Philippides G, Rocchini A. Council on Clinical Cardiology, American Heart Association Exercise, Cardiac Rehabilitation, and Prevention Committee; Council on Cardiovascular Disease in the Young; Council on Cardiovascular Nursing; Council on Nutrition, Physical Activity, and Metabolism; and Interdisciplinary Council on Quality of Care and Outcomes Research Exercise training for type 2 diabetes mellitus: impact on cardiovascular risk: a scientific statement from the American Heart Association. *Circulation*. 2009; 119(25):3244–3262. [PubMed: 19506108]
- Mimmack ML, Brooking J, Bahn S. Quantitative polymerase chain reaction: validation of microarray results from postmortem brain studies. *Biol Psychiatry*. 2004; 55:337–345. [PubMed: 14960285]
- Molteni R, Ying Z, Gómez-Pinilla F. Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. *Eur J Neurosci*. 2002; 16(6):1107–1116. [PubMed: 12383240]
- Neeper SA, Gomez-Pinilla F, Choi J, Cotman CW. Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Res*. 1996; 726(1–2):49–56. [PubMed: 8836544]
- Phillips HS, Hains JM, Laramée GR, Rosenthal A, Winslow JW. Widespread expression of BDNF, but not NT3 by target areas of basal forebrain cholinergic neurons. *Science*. 1990; 250(4978):290–294. [PubMed: 1688328]
- Rhyu IJ, Boklewski J, Ferguson B, Lee KJ, Lange H, Bytheway J, Cameron J. Exercise training is associated with increased cortical vascularization in adult female cynomolgus monkeys. *Soc Neurosci Abst*. 2003; 29:920–921.
- Shadoan MK, Anthony MS, Rankin SE, Clarkson TB, Wagner JD. Effects of tibolone and conjugated equine estrogens with or without medroxyprogesterone acetate on body composition and fasting carbohydrate measures in surgically postmenopausal monkeys. *Metabolism*. 2003; 52(9):1085–1091. [PubMed: 14506611]
- Stenvinkel P. C-reactive protein—does it promote vascular disease? *Nephrol Dial Transplant*. 2006; 21:2718–2720. [PubMed: 16861733]
- Steward O, Worley P. Local synthesis of proteins at synaptic sites on dendrites: role in synaptic plasticity and memory consolidation? *Neurobiol Learn Mem*. 2002; 78:508–527. [PubMed: 12559831]
- Stewart LK, Earnest CP, Blair SN, Church TS. Effects of different doses of physical activity on C-reactive protein among women. *Med Sci Sports Exerc*. 2009; 41(2):221–225.
- Sullivan EL, Cameron JL. A rapidly occurring compensatory decrease in physical activity counteracts diet-induced weight loss in female monkeys. *Am J Physiol Regul Integr Comp Physiol*. 2010; 298(4):R1068–R1074. [PubMed: 20071608]
- Sullivan EL, Koegler FH, Cameron JL. Individual differences in physical activity are closely associated with changes in body weight in adult female rhesus monkeys (*Macaca mulatta*). *Am J Physiol Regul Integr Comp Physiol*. 2006; 291(3):R633–R642. [PubMed: 16614060]

- Thacker EL, Chen H, Patel AV, McCullough ML, Calle EL, Thun MJ, Schwarzschild MA, Ascherio A. Recreational physical activity and risk of Parkinson's disease. *Mov Disord*. 2008; 23(1):69–74. [PubMed: 17960818]
- Tong L, Shen H, Perreau VM, Balazs R, Cotman CW. Effects of exercise on gene-expression profile in the rat hippocampus. *Neurobiol Dis*. 2001; 8(6):1046–1056. [PubMed: 11741400]
- Vance DE, Wadley VG, Ball KK, Roenker DL, Rizzo M. The effects of physical activity and sedentary behavior on cognitive health in older adults. *J Aging Phys Act*. 2005; 13(3):294–313. [PubMed: 16192657]
- Vazdarjanova A, Ramirez-Amaya V, Insel N, Plummer TK, Rosi S, Chowdhury S, Mikhael D, Worley PF, Guzowski JF, Barnes CA. Spatial exploration induces ARC, a plasticity-related immediate-early gene, only in calcium/calmodulin-dependent protein kinase II-positive principal excitatory and inhibitory neurons of the rat forebrain. *J Comp Neurol*. 2006; 498(3):317–329. [PubMed: 16871537]
- Volanakis JE, Kaplan MH. Specificity of C-reactive protein for choline phosphate residues of pneumococcal C-polysaccharide. *Proc Soc Exp Biol Med*. 1971; 136(2):612–614. [PubMed: 4395924]
- Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA. Decreased glutamic acid decarboxylase₆₇ messenger rRNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. *Arch Gen Psychiatry*. 2000; 57:237–245. [PubMed: 10711910]
- Wang Q, Zhu X, Xu Q, Ding X, Chen YE, Song Q. Effect of C-reactive protein on gene expression in vascular endothelial cells. *Am J Physiol Heart Circ Physiol*. 2005; 288:H1539–H1545. [PubMed: 15591095]
- Williams K, Sniderman AD, Sattar N, D'Agostino R Jr, Wagenknecht LE, Haffner SM. Comparison of the associations of apolipoprotein B and low-density lipoprotein cholesterol with other cardiovascular risk factors in the insulin resistance atherosclerosis study (IRAS). *Circulation*. 2003; 108(19):2312–2316. [PubMed: 14581403]
- Yaffe K, Barnes D, Nevitt M, Lui LY, Covinsky K. A prospective study of physical and cognitive decline in elderly women: women who walk. *Arch Intern Med*. 2001; 161(14):1703–1708. [PubMed: 11485502]
- Yeh ET. CRP as a mediator of disease. *Circulation*. 2004; 109(21):II11–II14. [PubMed: 15173057]
- Zhang HT, Li LY, Zou XL, Song XB, Hu YL, Feng ZT, Wang TT. Immunohistochemical distribution of NGF, BDNF, NT-3, and NT-4 in adult rhesus monkey brains. *J Histochem Cytochem*. 2007; 55(1):1–19. [PubMed: 16899765]

Fig. 1.

Correlation of spontaneous activity, serum CRP levels, and BDNF and ARC expressions in motor cortex of 11 rhesus monkeys. **a** Monkey activity levels were measured in counts/day, CRP in milligrams per liter (mg/L), BDNF and ARC in threshold cycle difference relative to β -actin (ΔC_t). **b** Scatter plot of ARC expression (*X*-axis) and BDNF expression (*Y*-axis) for the motor cortex of the 11 monkeys included in the study. *Solid line* trend line. Note the correlation between the expression of these two genes. **c** *X*-axis denotes gene expression level measured by qPCR for ARC (*blue diamonds*) and BDNF (*red squares*) for the 11 monkeys, while *Y*-axis represent end of experiment spontaneous activity counts. *Solid lines* trend lines. Note that both ARC and BDNF expressions are significantly correlated with

spontaneous activity in the studied monkeys. **d** Scatter plot of serum CRP levels (*X*-axis) versus spontaneous activity (*Y*-axis). Each *symbol* represents a single experimental animal. Note the inverse correlation between spontaneous activity and serum CRP levels. **e** Correlation of serum CRP level and ARC and BDNF expressions in the motor cortex. Figure layout similar to that of **c**. Note the high inverse correlation between the ARC–CRP and BDNF–CRP measurements