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Interleukin 6 deficiency affects spontaneous activity of mice in age- and sex-dependent manner

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We analyzed the role of interleukin 6 (IL-6) in modulation of the pattern of mice spontaneous activity. Wild type (WT) and IL-6 deficient mice of both sexes, young and aging, were housed individually and various types of their activity were recorded and analyzed with the Phenorack system in their home cages during 72 hours-long sessions. All investigated groups of mice were active mainly during the dark phase of the 24-hours cycle. Generally, the IL-6 deficient animals were more active than their WT controls and females of both genotypes more active than males. Aging mice were less active than the sex and genotype-matched young animals. The independent variables (age, sex and genotype) strongly interacted, which suggests that the modulatory influence of IL-6 on mice behavior may be different in males and females and that it changes during aging. We conclude that under normal physiological conditions signaling of IL-6 *via* its receptor participates in modulation of the basic pattern of activity. This modulation differs in males and females and changes with aging.

Key words: interleukin 6, spontaneous activity, aging, sex differences

INTRODUCTION

Interleukin 6 (IL-6) is a cytokine commonly known for proinflammatory functions, but accumulating evidence points it's pivotal role in the central nervous system physiology and pathology (for review, see Spooren et al. 2011). In recent years studies have shown that various cells of nervous tissue, including different brain structures and the cerebrospinal fluid of healthy subjects express IL-6 (Van Wagoner and Benveniste 1999, Carpenter et al. 2004, Lindqvist et al. 2009). The results from our laboratory indicate that in the brain the highest expression of IL-6 is present in astrocytes placed close to the border zone of the brain ventricles while its low level in neurons is observed in the hypothalamus, hippocampus, cerebral cortex, olfactory bulb and cerebellum (Aniszewska et al. 2014). Microglial cells also expressed IL-6 but its level is very low under normal physiological conditions. However, in numerous brain pathologies both neurodegenerative diseases and psychiatric disorders IL-6 levels become increased, which may be causative for the disease, but also could be considered as a side-effect of pathological processes not connected with etiology (Blum-Degen et al. 1995, Garver et al. 2003, Sun et al. 2003, Carpenter et al. 2004). In addition, the level of IL-6 expression increases not only in response to various stimuli, but also during normal aging processes. The level of IL-6 expression increases with age and susceptibility to this increase may affect longevity (Wei et al. 1992, Ershler et al. 2000, Bonafe et al. 2001, Goodbout and Johnson 2004). In healthy aged subjects elevated level of IL-6 may be a risk factor in subsequent cognitive decline (Weaver et al. 2002).

Studies evaluating influence of IL-6 on learning and memory in mice are less conclusive. In experiments performed by Baier and colleagues (2009) mice with IL-6 deficiency displayed impairments on hippocampus independent learning, as measured with novel object recognition memory test and in hippocampus dependent learning evaluated by Morris water maze. IL-6 deficient mice had significantly lower recognition index, which indicates that they were not able to distinguish between previously presented and novel objects.

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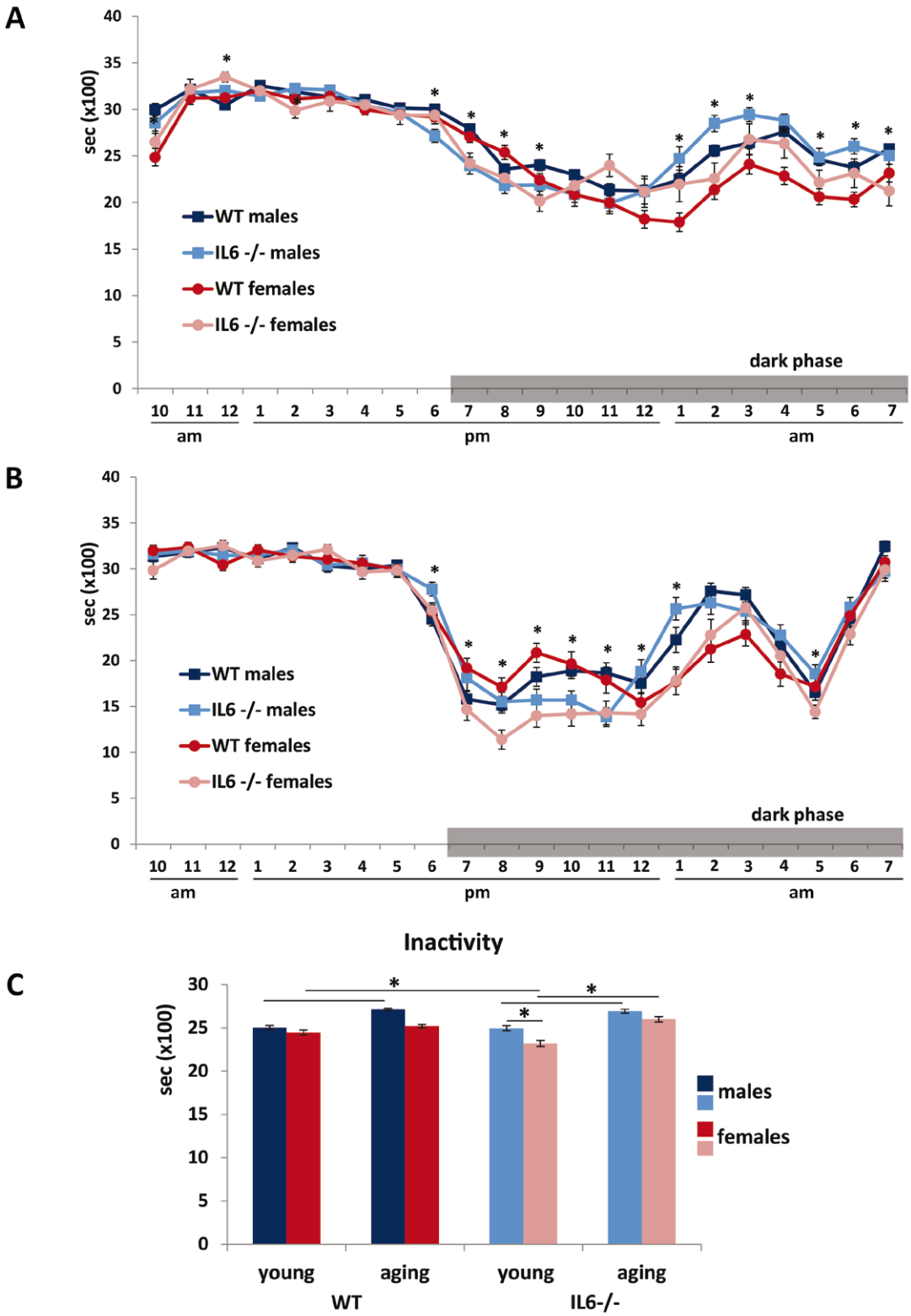

Fig. 1. Duration of inactivity (in seconds) during 22 hours of the recording period. Comparison of inactivity between aging (A) and young mice (B). Bars represent duration of inactivity during each hour of registration (average form three days). (C) comparison between experimental groups in inactivity during whole registration period (average from three days). Mean ± SEM). *P<0.05.

Additionally, in water maze their escape latency was significantly longer, than that of WT controls, in both the acquisition and reversal phases of the study (Baier et al. 2009). These findings are consistent with our unpublished results from Morris water maze, performed on identical, commercially available strain of IL-6 deficient mice. Interestingly, Braida and coworkers (2004) who used his independently created IL-6 deficient mice, found that they perform better than WT in the radial maze test with positive reinforcement. Percentage of animals reaching the criterion was significantly higher in IL-6 deficient animals, both young and aging, and the number of days needed to reach the criterion was higher in WT, but only in the aging group (Braida et al. 2004). One possible explanation of these discrepancies is that in the radial maze mice from all groups needed at least 15 days of acquisition to reach the criterion, whereas Baier and others (2009) tested mice after only two days of acquisition in the Morris water maze. In our unpublished study mice were trained in this test for 4 days, with test at the 5th day, therefore it is possible, that IL-6 influence may be too subtle to be revealed after short learning in this test and it showed only in case of longer learning. What more it is possible that using learning paradigm with positive reinforcement (like in Braida et al. 2004) may lead to different results, than Morris water maze test involving negative reinforcement.

IL-6 affects also emotionality and stress response (Kiecolt-Glaser et al. 2003, Chourbaji et al. 2006). It has been reported that IL-6 deficient mice displayed abnormal behavior (Armario et al. 1998, Butterweck et al. 2003). They spent less time in the open arms of the plus maze and the number of entries there is lower compared to wild type (WT) mice. These data suggest that IL-6 deficient mice show the reduced level of exploratory activity. Administration of IL-6 also alter behavior of mice increasing exploratory and locomotor activity (Zalcman et al. 1998). Interestingly, different strains of mice exhibit different locomotor activity, even mice from inbred strains differ individually (Tang et al. 2002, Tang and Sanford 2005, Zarringhalam et al. 2012, Aniszewska et al. 2014). Based on these data we hypothesize that IL-6 could be involved in the spontaneous locomotor activity of mice.

In this study we investigated the spontaneous locomotor activity of IL-6 deficient mice and tested whether IL-6 involves locomotor activity of young and age mice of both sexes.

METHODS

Animals

Experiments were performed on 81 wild-type (WT) C57BL6/J and 58 IL-6 deficient mice (IL6-/- tmKopf on C57BL/6 background) of both sexes. Young mice at 3 months old and aging mice at 10–16 months old were used. Mice were kept in quiet, separate room, at 23°C and a 12-hour light-dark cycle. Food and water were available *at libitum*. Experimental procedures complied with the Polish Law on Experimentation on Animals that implements the European Council Directive of 24 November 1986 (86/609/EEC) and the NIH Guide for the Care and Use of Laboratory Animals. The experiments were approved and controlled by a local ethics committee in Warsaw.

Spontaneous activity recordings

To record and analyze spontaneous activity of mice in their home cages we used the PhenoRack system (ViewPoint Life Sciences, Inc.). There were four animals recorded at the same time in separate cages.

Each of the four standard home cages (20×36 cm) was placed between infrared illuminators and CCD infrared video cameras allowing for the recording of activity during both light and dark phases of the circadian cycle.

Before the start of recording, mice were housed individually in cages for 72 hours (habituation to solitary housing) and then the cages were placed in the system for 24 hours (habituation to recording environment). Afterwards, the behavior of each mouse was registered during the following 3 days. Each day, the recording was interrupted for 2 hours (from 08:00 AM to 10:00 AM) to allow for the maintenance of the cages and room by animal service. Cages containing mice during ongoing observation were not interrupted.

The active and passive behaviors of the mice were automatically quantified with the PhenoRack software. During the inactive period, mice slept and did not move most of the time, except for some slow movement prior to sleep. When mice were awake, they moved about the cage, ate and drank water; we refer to this as moderate activity. When the mice were running, jumping, digging or hanging from the top mesh, we classified their behavior as rapid movement and burst activity.

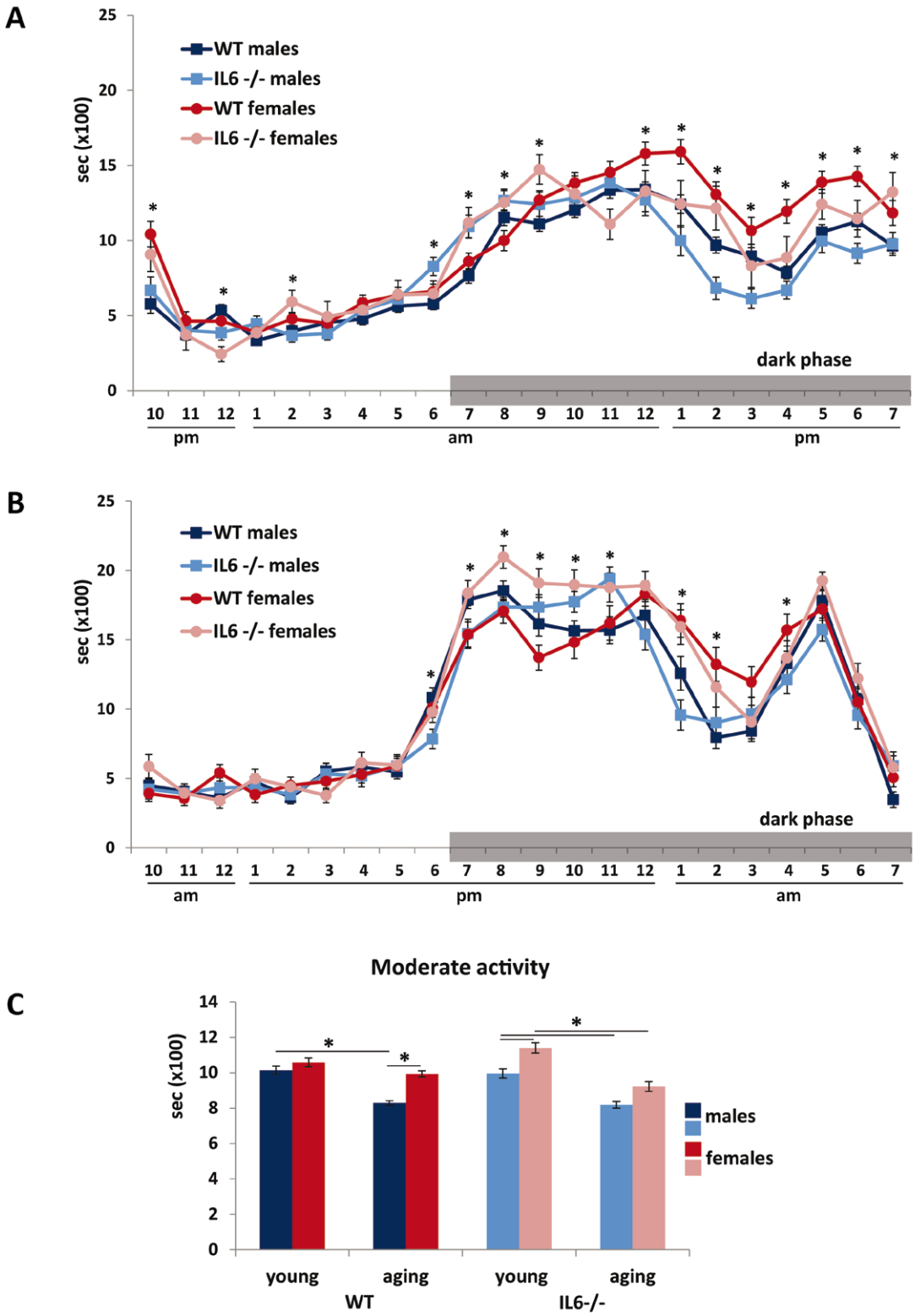

Fig. 2. Duration of moderate activity (in seconds) during 22 hours of the recording period. Comparison of moderate activity between aging (A) and young mice (B). Bars represent duration of moderate activity during each hour of registration (average form three days). (C) comparison between experimental groups in moderate activity during whole registration period (average from three days). Mean ± SEM). *P<0.05.

Statistical analysis

Behavioral data were analyzed using two or three-way ANOVA followed by *post-hoc* multiple pair-wise comparisons using the Bonferroni test. Independent variables were: sex (males and females), age (young vs. aging mice) and genotype (WT vs. IL-6 deficient). The statistical significance level was set at $P<0.05$.

RESULTS

During the 3-day-long test we investigated the behavior of WT and IL-6 deficient, young and aging mice of both sexes in their home cages. We found that during daytime all mice were inactive and slept for most of the time. Starting from 10:00 AM until 06:00 PM (light phase), mice were motionless for approximately 80–85% of each hour (Fig. 1A, B). On the contrary, in the dark phase all mice were active for long periods of time. We evaluated two types of locomotor activity using the PhenoRack software. Grooming, eating or walking were classified as a moderate locomotor activity, while running, jumping or digging were assigned to the rapid or burst activity. We observed a bimodal curve of activity in the first few hours of the dark phase and found that the majority of significant differences between young and aging mice occurred at the dark phase (Fig. 2A, B).

The results quantification of inactivity, moderate activity and burst activity reveal similar patterns, although differences between experimental groups were most distinct for burst activity.

The three-way ANOVA was used to compare three different parameters of behavior (inactivity, moderate activity and burst activity) for three independent factors: genotype, sex and age. There was no significant difference between WT and IL-6 deficient mice in parameters of inactivity and moderate activity, while the IL-6 deficient mice engaged in significantly more burst activity than WT animals ($F_{1,9055}$=55.320, $P<0.0001$).

The same ANOVA analysis confirmed significant differences between sexes for each of the behavioral parameters. Female mice were inactive for smaller proportion of time than males ($F_{1,9055}$= 50.949, $P<0.0001$) and spent more time on both moderate activity ($F_{1,9055}$=53.243, $P<0.0001$) and burst activity ($F_{1,9055}$= 24.049, $P<0.0001$).

Aging mice were generally less active than young animals. They spent more time being inactive ($F_{1,9055}$=110.415, $P<0.0001$), and less time being moderately active ($F_{1,9055}$=105.980, $P<0.0001$) or exhibiting burst activity ($F_{1,9055}$=90.541, $P<0.0001$).

Aside from the main effects, some of the interactions between genotype, sex and age were significant. We found significant interactions of genotype and age for the parameters of inactivity ($F_{1,9055}$=7.074, $P<0.008$), moderate activity ($F_{1,9055}$=5.388, $P<0.02$) and burst activity $F_{1,9055}$=14.960, $P<0.0001$). The *post-hoc* analysis confirmed that in both WT and IL-6 deficient mice young animals were more active than aging ones ($P<0.0001$; Figs 1–3). Interaction of sex and age for the parameters of inactivity and moderate activity was not significant, however there was a strong interaction for the burst activity parameter ($F_{1,9055}$=5.399, $P<0.02$). Interactions of genotype and sex were not significant for any of the three parameters of activity.

Finally, interaction of genotype, age and sex was significant for inactivity ($F_{1,9055}$=9.163, $P<0.002$), moderate activity ($F_{1,9055}$=6.658, $P<0.01$) and burst activity ($F_{1,9055}$=22.196, $P<0.0001$) parameters. Generally, females were more active than males (main effect of sex) but the *post hoc* analysis revealed that this effect was not observed in young WT and in aging IL-6 deficient mice (there were no significant differences between males and females in these groups). Additionally, the differences between young and aging mice are more profound in WT males than females, unlike in the IL-6 deficient mice, where differences between young and aging mice were clear in females, while males behaved similarly (especially when the parameter of burst activity was compared, Fig. 3C). Thus, sex differences were distinct in the aging WT and young IL-6 deficient mice. This result indicates, that the influence of IL-6 on mice activity is strongly age- and sex-dependent.

DISCUSSION

In the present study we evaluated the role of IL-6 in the spontaneous locomotor activity of young and aging mice. Because of well-known sex differences in mice brain and behavior we performed experiments on males and females (Chłodzińska et al. 2011, McCarthy et al. 2012). We found that the spontaneous activity varies between males and females and between young and aging animals. Consistently with previous data, we observed intensive activity of mice in the dark phase and females were more active than males

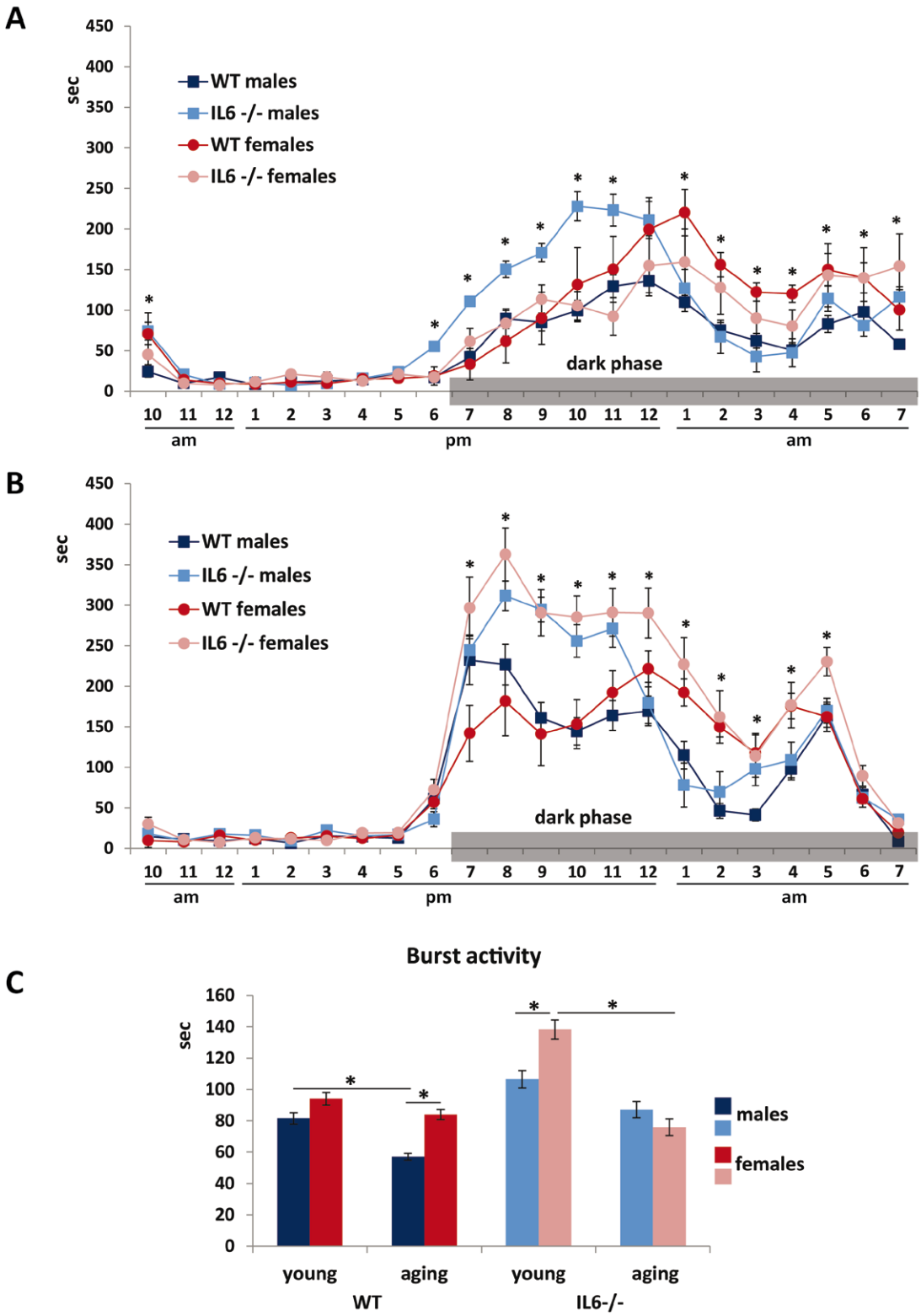

Fig. 3. Duration of burst activity (in seconds) during 22 hours of the recording period. Comparison of burst activity between aging (A) and young mice (B). Bars represent duration of burst activity during each hour of registration (average form three days). (C) comparison between experimental groups in burst activity during whole registration period (average from three days). Mean ± SEM). *P<0.05.

(Aniszewska et al. 2014). On the contrary, in most behavioral experiments locomotor activity (performed usually in the open field test and presented as total distance travelled in the field) indicates that males are usually more active/less anxious than females, or there is no sex dependent differences (An et al. 2011, Huynh et al. 2011, Ngun et al. 2011, Lin et al. 2011, Van Swearingen et al. 2013, Aniszewska et al. 2014). This inconsistency suggests that results of locomotor activity measures based on the open field test are strongly affected by stress or anxiety.

As a component of immune system IL-6 undergoes processes comprising the immunosenescence, which are strongly sex-dependent (Caruso et al. 2013, Verdecia et al. 2013). It has been shown that there are differences in immune function between males and females (Scotland et al. 2011, Oertelt-Prigione 2012, Pennell et al. 2012). Generally, females developed stronger proinflammatory response than males in response to immune stimulus, which can be beneficial, because of potentially faster pathogen clearance, but it can also be detrimental, due to increased susceptibility to autoimmune diseases in females (Klein 2012). We found that young animals were more active than aging, and this effect was profound especially in IL-6 deficient females. Additionally, aging WT females were more active than males while this effect was not observed in aging IL-6 deficient mice. It suggests that IL-6 influence on mice activity is different in aging mice, especially in aging females, which is consistent with findings regarding sex differences of cytokine levels due to aging processes that lead to increased proinflammatory status in the brain and the periphery (Miller et al. 2010, Gano et al. 2011, Song et al. 2012, Villar-Cheda et al. 2012).

The effects of estradiol, a female sex hormone actions on behavior have been extensively explored (Barha and Galea 2010, Morgan and Pfaff 2002, Morgan et al. 2004). Estradiol has been shown to affect among others anxiety and fear related behaviors. Ovariectomized female mice treated with estrogen exhibited increased level of anxiety measured in the open field and elevated plus maze test. Interestingly, estradiol treatment led to increased spontaneous locomotor activity of mice in their home cages (Morgan and Pfaff 2001). This finding suggests that estradiol interacts with environmental factors in controlling behavior. In familiar, non-threatening environment, estradiol induces high activity, which explains higher spontaneous locomotor activity in females, observed in our study. In the brain estrogen is known to act as antiinflammatory and neuroprotective factor (Arevalo et al. 2010). Estrogen receptors are able to repress the expression of many proinflammatory cytokines, including IL-6 (Galien and Garcia 1997, Liu et al. 2005). It is still not thoroughly investigated whether and how estradiol level changes in the aging brain. On the contrary to estradiol actions, based on observations that an elevated level of IL-6 may lead to decreased activity, we hypothesize that spontaneous activity of female mice can be affected by both estradiol and IL-6. In young and healthy WT animals estradiol and IL-6 determine the basic level of activity, whereas in aging animals estradiol level decreases and IL-6 level increases, which leads to a reduction of their spontaneous activity. This effect is less profound in IL-6 deficient females, because lack of IL-6 expression (and age related increase). Correspondingly, in males, where estradiol level is relatively constant, only an influence of IL-6 is observed (slightly higher levels of spontaneous activity in IL-6 deficient males and its decrease due to aging).

Recent findings have indicated that IL-6 directly affects learning and memory processes, as well as anxiety-driven behavior (Armario et al. 1998, Braida et al. 2004, Baier et al. 2009, Spooren et al. 2011). IL-6 cross the blood-brain barrier, connects the brain and the immune system, carrying the information about the inflammatory status from the periphery to the brain (Banks and Erickson 2010). Therefore, both IL-6 produced by brain cells and peripherally derived IL-6 cells are involved in some brain functions. Additionally, Jarskog and coworkers (1997) have reported that IL-6 regulates survival of the fetal dopamine and serotonin neurons *in vitro*. We suggest that IL-6 through modulation of dopaminergic and serotoninergic systems affects the behavior of adult animals. Experiments performed on mice with IL-6 deletion targeted only in brain cells can give answers to this question.

CONCLUSION

We conclude that IL-6 plays an important role in basic behavioral patterns and that this influence can be different in males compared to females and can be modified across the lifespan.

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REFERENCES

Armario A, Hernandez J, Bluethmann H, Hidalgo J (1998) IL-6 deficiency leads to increased emotionality in mice: evidence in transgenic mice carrying a null mutation for IL-6. J Neuroimmunol 92: 160–169.

An X-L, Zou J-X, Wu R-Y, Yang Y, Tai F-D, Zeng S-Y, Jia R, Liu E-Q, Broders H (2011) Strain and sex differences in anxiety-like and social behaviors in C57BL/6J and BALB/cJ mice. Exp Anim 60: 111–123.

Aniszewska A, Chłodzińska N, Bartkowska K, Winnicka MM, Turlejski K, Djavadian RL (2014) The expression of interleukin-6 and its receptor in various brain regions and their roles in exploratory behavior and stress responses. J Neuroimmunol (in press).

Arevalo MA, Santos-Galindo M, Bellini MJ, Azcoitia J, Garcia-Segura LM (2010) Actions of estrogens on glial cells: Implications for neuroprotection. Biochim Biophys Acta 1800: 1106–1112.

Baier PC, May U, Scheller J, Rose-John S, Schiffelholz T (2009) Impaired hippocampus-dependent and- independent learning in IL-6 deficient mice. Behav Brain Res 200: 192–196.

Banks WA, Erickson MA (2010) The blood–brain barrier and immune function and dysfunction. Neurobiol Dis 37: 26–32.

Barha CK, Galea LAM (2010) Influence of different estrogens on neuroplasticity and cognition in the hippocampus. Biochim Biophys Acta 1800: 1056–1067.

Blum-Degen D, Mtiller T, Kuhn W, Geflach M, Przuntek H, Riederer P (1995) Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. Neurosci Lett 202: 17–20.

Bonafe M, Olivieri F, Cavallone L, Giovagnetti S, Marchegiani F, Cardelli M, Pieri C., Marra M, Antonicelli R, Lisa R., Rizzo M.R, Paolisso G, Monti D, Franceschi C (2001) A gender–dependent genetic predisposition to produce high levels of IL-6 is detrimental for longevity. Eur J Immunol 31: 2357–2361.

Braida D, Sacerdote P, Panerai AE, Bianchi M, Aloisi AM, Iosue M, Sala M (2004) Cognitive function in young and adult IL (interleukin)-6 deficient mice. Behav Brain Res 153: 423–429.

Butterweck V, Prinz S, Schwaninger M (2003) The role of interleukin-6 in stress-induced hyperthermia and emotional behaviour in mice. Behav Brain Res 144: 49–56.

Carpenter LL, Heninger GR, Malison RT, Tyrka AR, Price LH (2004) Cerebrospinal fluid interleukin (IL)-6 in unipolar major depression. J Affect Disord 79: 285–289.

Caruso C, Accardi G, Virruso C, Candore G (2013) Sex, gender and immunosenescence: a key to understand the different lifespan between men and women? Immun Ageing 10: 20.

Chłodzińska N, Gajerska M, Bartkowska K, Turlejski K, Djavadian RL (2011) Lipopolysaccharide injected to pregnant mice affects behavior of their offspring in adulthood. Acta Neurobiol Exp (Wars) 71: 519–527.

Chourbaji S, Urani A, Inta J, Sanchis-Segura C, Brandwein C, Zink M, Schwaninger M, Gass P (2006) IL-6 knockout mice exhibit resistance to stress-induced development of depression-like behaviors. Neurobiol Dis 23: 587–594.

Ershler WB, Keller ET (2000) Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med 51: 245–270.

Galien R, Garcia T (1997) Estrogen receptor impairs interleukin-6 expression by preventing protein binding on the NF-kB site. Nucleic Acids Res. 25(12): 2424-9.

Gano LB, Donato AJ, Pierce GL, Pasha HM, Magerko KA, Roeca C ,Seals DR (2011) Increased proinflammatory and oxidant gene expression in circulating mononuclear cells in older adults: amelioration by habitual exercise. Physiol Genomics 43: 895–902.

Garver DL, Tamas RL, Holcomb JA (2003) Elevated Interleukin-6 in the cerebrospinal fluid of a previously delineated schizophrenia subtype. Neuropsychopharmacology 28: 1515–1520.

Goodbout JP, Johnson RW (2004) Interleukin-6 in the aging brain. J Neuroimmunol 147: 141–144.

Huynh TN, Krigbaum AM, Hanna JJ, Conrad CD (2011) Sex differences and phase of light cycle modify chronic stress effects on anxiety and depressive-like behavior. Behav Brain Res 222: 212–222.

Jarskog LF, Xiao H, Wilkie MB, Lauder JM, Gilmore JH (1997) Cytokine regulation of embryonic rat dopamine and serotonin neuronal survival *in vitro*. Int J Dev Neurosci 15: 711–716.

Kiecolt-Glaser JK, Preacher KJ, MacCallum RC, Atkinson C, Malarkey WB, Glaser R (2003) Chronic stress and age-related increases in the proinflammatory cytokine IL-6. Proc Natl Acad Sci U S A 100: 9090–9095.

Klein SL (2012) Immune cells have sex and so should journal articles. Endocrinology 153: 2544–2550.

Lin E-JD, Choi E, Liu X, Martin A, During MJ (2011) Environmental enrichment exerts sex-specific effects on emotionality in C57BL/6J mice. Behav Brain Res 216: 349–357.

Lindqvist D, Janelidze S, Hagell P, Erhardt S, Samuelsson M, Minthon L, Hansson O, Björkqvist M, Träskman-Bendz L, Brundin L (2009) Interleukin-6 is elevated in the cerebrospinal fluid of suicide attempters and related to symptom severity. Biol Psychiatry 66: 287–292.

Liu H, Liu K, Bodenner DL (2005) Estrogen receptor inhibits interleukin-6 gene expression by disruption of nuclear factor kB transactivation. Cytokine 31: 251–257.

McCarthy MM, Arnold AP, Ball GF, Blaustein JD, De Vries GJ (2012) Sex Differences in the Brain: The Not So Inconvenient Truth. J Neurosci 32: 2241–2247.

Miller VM, Lawrence DA, Coccaro GA, Mondal TK, Andrews K, Dreiem A, Seegal RF (2010) Sex effects of Interleukin-6 deficiency on neuroinflammation in aged C57Bl/6. Brain Res 1318: 11–22.

Morgan MA, Pfaff DW (2001) Effects of estrogen on activity and fear-related behaviors in mice. Horm Behav 40: 472–482.

Morgan MA, Pfaff DW (2002) Estrogen's effects on activity, anxiety, and fear in two mouse strains. Behav Brain Res 132: 85–93.

Morgan MA, Schulkin J, Pfaff DW (2004) Estrogens and non-reproductive behaviors related to activity and fear. Neurosci Biobehav Rev 28: 55–63.

Ngun TC, Ghahramani N, Sánchez FJ, Bocklandt S, Vilain E (2011) The genetics of sex differences in brain and behavior. Front Neuroendocrinol 32: 227–246

Oertelt-Prigione S (2012) The influence of sex and gender on the immune response. Autoimmun Rev 11: 479–485.

Pennell LM, Galligan CL, Fish EN (2012) Sex affects immunity. J Autoimmun 38: 282–291.

Scotland RS, Stables MJ, Madalli S, Watson P, Gilroy DW (2011) Sex differences in resident immune cell phenotype underlie more efficient acute inflammatory responses in female mice. Blood 118: 5918–5927.

Song Y, Shen H, Schenten D, Shan P, Lee PJ, Goldstein DR (2011) Aging enhances the basal production of IL-6 and CCL2 in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 32: 103–109.

Spooren A, Kolmus K, Laureys G, Clinckers R, De Keyser J, Haegeman G, Gerlo S (2011) Interleukin-6, a mental cytokine. Brain Res Rev 67: 157–183.

Sun YX, Minthon A, Wallmark S, Warkentin K, Blennow, Janciauskiene S (2003) Inflammatory markers in matched plasma and cerebrospinal fluid from patients with Alzheimer's disease. Dement Geriatr Cogn Disord 16: 136–144.

Tang X, Sanford LD (2005) Home cage activity and activity-based measures of anxiety in 129P3/J, 129X1/SvJ and C57BL/6J mice. Physiol Behav 84: 105–115.

Tang X, Orchard SM, Sanford LD (2002) Home cage activity and behavioral performance in inbred and hybrid mice. Behav Brain Res 136: 555–569.

Van Swearingen AED, Walker QD, Kuhn CM (2013) Sex differences in novelty- and psychostimulant-induced behaviors of C57BL/6 mice. Psychopharmacology (Berl) 225: 707–718.

Van Wagoner NJ, Benveniste EN (1999) Interleukin-6 expression and regulation in astrocytes. J Neuroimmunol 100: 124–139.

Verdecia BG, Hernández DS, Lorenzo-Luaces P, Alvarez TJB, Rupalé IL, Herrera ZM, Ramos TC, Dávila AL (2013) Immunosenescence and gender: a study in healthy Cubans. Immun Ageing 10: 16.

Villar-Cheda B, Valenzuela R, Rodriguez-Perez AI, Guerra MJ, Labandeira-Garcia JL (2012) Aging-related changes in the nigral angiotensin system enhanced proinflammatory and pro-oxidative markers and 6-OHDA-induced dopaminergic degeneration. Neurobiol Aging 33: 204.1–204.11.

Weaver JD, Huang MH, Albert M, Harris T, Rowe JW, Seeman TE (2002) Interleukin-6 and risk of cognitive decline. Neurology 59: 371–378.

Wei J, Xu H, Davies JL, Hemmings GP (1992) Increase of plasma IL-6 concentration with age in healthy subjects. Life Sci 51: 1953–1956.

Zalcman S, Murray L, Dyck DG, Greenberg AH, Nance DM (1998) Interleukin-2 and -6 induce behavioral-activating effects in mice. Brain Res 811: 111–121.

Zarringhalam K, Ka M, Kook Y, Terranova JI, Suh Y (2012) An open system for automatic home-cage behavioral analysis and its application to male and female mouse models of Huntington's disease. Behav Brain Res 229: 216–225.