Biogerontology

Depression-like behavior in senescence accelerated P8 mice

--Manuscript Draft--

Manuscript Number:	BGEN-D-13-00020R2
Full Title:	Depression-like behavior in senescence accelerated P8 mice
Article Type:	Research Article
Section/Category:	Research Article
Keywords:	Aging, Alzheimer's disease, Depression, Forced Swimming Test, Dexamethasone Suppression Test, SAMP8
Corresponding Author:	Merce Pallas Barcelona, SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	
Corresponding Author's Secondary Institution:	
First Author:	David Pérez-Cáceres
First Author Secondary Information:	
Order of Authors:	David Pérez-Cáceres
	Andrés Ciudad
	M Teresa Rodrigo
	David Pubill
	Antoni Camins
	Jorge Camarasa
	Elena Escubedo
	Merce Pallas
Order of Authors Secondary Information:	
Abstract:	Aging is associated with an increased risk of depression in humans. To elucidate the underlying mechanisms of depression and its dependence on aging, here we study signs of depression in SAMP8 mice. For this purpose, we used the forced swimming test (FST). The total floating time in the FST was greater in SAMP8 than in SAMR1 mice at 9 months of age; however, this difference was not observed in 12-month-old mice, when both strains are considered elderly. Of the two strains, only the SAMP8 animals responded to imipramine treatment. We also applied the dexamethasone suppression test (DST) and studied changes in the dopamine (DA) and serotonin (5-HT) uptake systems, the 5-HT2a/2c receptor density in the cortex, and levels of TPH2. The DST showed a significant difference between SAMR1 and SAMP8 mice at old age. SAMP8 exhibits an increase in 5-HT transporter density, with slight changes in 5-HT2a/2c receptor density. In conclusion, SAMP8 mice presented depression-like behavior that involved monoamine levels.
Response to Reviewers:	Barcelona, March 21th, 2013 Dear Editor, Thank you for giving us this opportunity to submit a revised version of our manuscript. We have change the names to the Biogerontology style and we formatted references - citation and listing - should be in accordance with the style of Biogerontology

	Sincerely yours Dra. Mercè Pallàs Tenure professor Faculty of Pharmacy University of Barcelona, Spain

Title:

Depression-like behavior is depending on age in male SAMP8 mice

Authors:

D. Pérez-Cáceres[b], A. Ciudad-Roberts[a], M.T. Rodrigo[b], D. Pubill[a], A. Camins[a,c], J. Camarasa[a], E. Escubedo[a]✉, M. Pallàs[a,c]

Affiliation:

[a]Department of Pharmacology and Therapeutic Chemistry (Pharmacology Section) and Institute of Biomedicine (IBUB), University of Barcelona, Avda Joan XXIII s/n. 08028 Barcelona, Spain

[b]Animal Experimentation Unit of Psychology, University of Barcelona, Campus Mundet. Pg. Vall d'Hebron 171. Edifici de Ponent. 08035 Barcelona, Spain

[c]Centros de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED). Spain

✉ Dra. Elena Escubedo, PhD.

Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia

Universitat de Barcelona, Nucli Universitari de Pedralbes, E-08028 Barcelona, Spain

eescubedo@ub.edu. Tel: (+34) 934 024 531 ext 27. Fax: (+34) 934 035 982

Abstract

Aging is associated with an increased risk of depression in humans. To elucidate the underlying mechanisms of depression and its dependence on aging, here we study signs of depression in male SAMP8 mice. For this purpose, we used the forced swimming test (FST). The total floating time in the FST was greater in SAMP8 than in SAMR1 mice at 9 months of age; however, this difference was not observed in 12-month-old mice, when both strains are considered elderly. Of the two strains, only the SAMP8 animals responded to imipramine treatment. We also applied the dexamethasone suppression test (DST) and studied changes in the dopamine (DA) and serotonin (5-HT) uptake systems, the 5-$HT_{2a/2c}$ receptor density in the cortex, and levels of TPH2.

The DST showed a significant difference between SAMR1 and SAMP8 mice at old age. SAMP8 exhibits an increase in 5-HT transporter density, with slight changes in 5-$HT_{2a/2c}$ receptor density. In conclusion, SAMP8 mice presented depression-like behavior that is depending on senescence process, because differs from SAMR1, senescence resistant strain.

Keywords: Aging, Depression, Forced Swimming Test, Dexamethasone Suppression Test, SAMP8.

1. INTRODUCTION

Aging is associated with increased depression in humans. Although this condition is one of the most prevalent psychopathologies worldwide (Murray and López 1997), it is often not recognized or treated. The pathophysiology of depression has been associated with dysfunction in dopamine (DA) and serotonin (5-HT) neurotransmission (Nutt 2006). Age also brings changes in 5-HT and DA levels in humans in certain neurodegenerative diseases (Gareri et al 2002; Potter et al 2012; Gourley and Taylor 2009, Ownby et al 2006). Moreover, the levels of D2 and D1 receptors in suicidal individuals remain unchanged, while the turnover of DA is modified. Although the serotonergic system has many components, the serotonin transporter (SERT), the serotonin 1a receptor ($5\text{-}HT_{1a}$), and the serotonin 2a receptor ($5\text{-}HT_{2a}$) are those most closely associated with the neurobiology of mood.

The most highly prescribed medications for depression are 5-HT selective reuptake inhibitors that alter SERT protein levels and also its mRNA levels (Smith 1991; Owens and Nemeroff 1998). Depressed patients show a reduction in 5-HT metabolites in the serotonergic system. The involvement of $5\text{-}HT_{1a}$ receptors in human depression has been demonstrated, as their concentrations are markedly lower in depressed people than in people who are not depressed. Many electrophysiological studies have shown that antidepressants upregulate or "sensitize" $5\text{-}HT_{1a}$ function in the hippocampus, while at the same time downregulating or "desensitizing" $5\text{-}HT_{1a}$ function in the raphe, where this neurotransmitter acts as an inhibitory somatodendritic receptor Blier and de Montigny 1994). The $5\text{-}HT_{2a}$ receptor has also been shown to be affected by chronic antidepressant treatment. Most, but not all, studies have reported decreases in $5\text{-}HT_{2a}$ binding in the prefrontal cortex after chronic antidepressant administration Bourin and Baker 1996; Peroutka and Snyder 1980). The opposite changes ($5\text{-}HT_{2a}$ upregulation) have been reported in the prefrontal cortex of suicide victims Mann et al 1986; Arango et al 1990), although these findings are not universal (Stockemeier 1997). In addition, subjects with a history of depression who die of natural causes have been shown to present increases in $5\text{-}HT_{2a}$ binding in the prefrontal cortex (Yates et al 1990). There is accumulating genetic and neurobiological evidence of the critical role of tryptophan hydroxylase-2 (TPH2), the key enzyme for synthesis of 5-

HT, in central serotonergic system function and in the pathophysiology of a wide spectrum of disorders affecting cognitive control and emotion regulation, ranging from depression to attention-deficit/hyperactivity disorder (Waider et al 2011). Several studies drawn also the implication of BDNF in depression and has received considerable support the functional Val66Met polymorphism of the gene encoding BDNF, which may reduce BDNF expression particularly when exposed to stress and thus may play a critical role in the pathogenesis of depression Groves 2007; Kimpton 2012).

The senescence accelerated mouse (SAM), a murine model of aging, is generated from autogenic senile strains of mice (Takeda et al 1981; 1991). There are nine senescence-prone strains (SAMP) and three senescence-resistant one (SAMR). SAMR strains are resistant to early senescence and are used as controls. Among the SAMP strains, SAMP8 and SAMP10 exhibit deficits in learning and memory at relatively early stages of life. SAMP8 mice also produce increased amounts of amyloid precursor protein (APP) and amyloid-β similar to those observed in Alzheimer's disease (AD) patients (Butterfield and Poon 2005) and spontaneous overproduction of soluble Aβ in the hippocampus and cortex (Petursdottir et al 2007; Gutierrez-Cuesta et al 2008). Therefore this strain may be a suitable model with which to study the fundamental mechanisms of age-related alterations in the central nervous system (CNS) and to evaluate the effects of drugs.

There is little evidence that the SAM strains show the symptoms of depression. Furthermore, studies suggest that SAMP8 mice exhibit an age-related emotional disorder characterized by reduced anxiety-like behavior Miyamoto et al 1992). Tests of learning strategies have demonstrated differences in 5-HT_1 and 5-HT_2 receptor activity in 12- and 4-month-old SAMP8 animals (Flood et al 1998).

Here we studied signs of depression in male SAMP8 mice in order to elucidate the underlying mechanisms of depression and its dependence on aging. Firstly, male SAMP8 and SAMR1 mice were subjected to the forced swimming test. We also tested reversion due to imipramine treatment in both strains. Finally, as markers of the neurobiological and anatomical basis of depression, the dexamethasone suppression test (DST) and changes in the DA and 5-HT uptake systems, 5-$HT_{2a/2c}$ receptor density in the cortex and TPH2 protein levels were studied in male SAMP8 and SAMR1.

2. METHODS

2.1.Animals

The experimental protocols for the use of animals in this study were approved by the Animal Ethics Committee of the University of Barcelona under the supervision of the Autonomous Government of Catalonia, following the guidelines of the European Communities Council (86/609/EEC). Efforts were made to minimize suffering and reduce the number of animals used. Male 6-, 9- and 12-month-old SAMR1 and SAMP8 mice were used in all experiments, which were housed at 22 ± 1 ºC under a 12-h light/dark cycle with free access to food and drinking water. Mean lifespan for SAMR1 are 16.3 months and for SAMP8 are 9.7 months).

2.2.Forced Swimming Test

To conduct the forced swimming test (FST), animals (9 to 14 different mice for each experimental group) were placed in a Plexiglas cylinder (10 cm internal diameter, 50 cm high) filled with water (10 cm height) at 22–25 °C. Duration of the experiment was 5 min; the behavior of the animals was evaluated during 3 min between the 2^{nd} and 5^{th} minutes and immobility time was measured. A mouse was judged to be immobile when it remained floating in the water, making only those movements necessary to keep its head above the water (Porsolt 2000; Porsolt et al 1977). The experiment was carried out before and after the imipramine treatment regime (30mg/kg IP, once a day for 7 days).

CompuLet 5 system (Panlab SL, Barcelona, Spain) was used to quantify the changing state of mobility to immobility (named as event) and also the time spent in each event (sec/event). For each experimental group events were expressed as the median ± semi-interquartile range, and statistically significant changes were analyzed with a Kruskal-Wallis test for non- parametric data, followed by U-Mann Whitney test. The difference between groups in the immobility time for each event was calculated using two-way ANOVA.

2.3.Dexamethasone suppression test

The dexamethasone suppression test was performed in male SAMR1 and SAMP8 mice (6-,9- and 12-month-old, n=9-10 for each experimental group). A blood sample was collected between 18:00 and 20:00 hours from separate cohorts of mice at baseline or after injection of one dose of dexamethasone (6µg/kg i.p. diluted in

saline). Blood samples were collected through the facial vein 8-9h after injection in microtubes containing K_3-EDTA. The samples were centrifuged at 1000 x *g* for 10min at 4°C; plasma were collected and stored at −80°C until corticosterone concentrations were assessed with a radioimmunoassay (^{125}I RIA Kit Immunchem Double Antibody Corticosterone, MP Biomedical LLC).The radioimmune assay was conducted following the manufacturer instructions. This assay is highly specific, cross reacting at less than 0.35% with other hormones and a detection limit of 7.7 ng/mL. The standard curve was run in triplicate and samples were run in duplicate. All samples within an experiment were run in the same assay. Results were expressed as the difference in the corticosterone levels before and after administration of the drug. Statistically significant changes were analyzed using a two-way ANOVA followed by Tukey post-hoc tests

2.4.Determination of transporters and receptors

2.4.1. Tissue sample preparation

SAM-P8 and SAM-R1 mice 6-,9- and 12 month-old (n=6 different mice for each experimental group) were killed by cervical dislocation. Immediately after sacrifice, they were decapitated and the brains rapidly removed from the skull. Striata, parietal cortex, frontal cortex and hippocampus were quickly dissected out, frozen on dry ice and stored at -80ºC until use. When required, tissue samples were thawed and homogenized at 4 ºC in 10 volumes of buffer consisting of 5 mM Tris-HCl, 320 mM sucrose and protease inhibitors (aprotinin 4.5 µg/µl, 0.1 mM phenylmethylsulfonyl fluoride and 1 mM sodium orthovanadate), pH 7.4, with a Polytron homogenizer. The homogenates were centrifuged at 1,000 x *g* for 15 min at 4^{o}C. Aliquots of the resulting supernatants were taken, and after the protein concentration was determined, they were frozen and kept for Western blot experiments. The pellets were resuspended with the remaining supernantants and centrifuged at 15,000 x *g* for 30 min at 4^{o}C. The pellets were resuspended in fresh buffer and incubated at 37 ºC for 10 min in order to remove endogenous neurotransmitters. The protein samples were then re-centrifuged and washed two more times. The final pellets (crude membrane preparation) were resuspended in the appropriate buffer and stored at -80ºC for use in radioligand binding experiments. Protein content was determined

using the Bio-Rad Protein Reagent (Bio-Rad Labs. Inc., Hercules, CA, USA),according to the manufacturer's specifications.

2.4.2.Western blotting and immunodetection

For immunodetection procedures, 20 µg of protein was separated by SDS–PAGE (5-15%) and transferred to PVDF membranes (Millipore). The membranes were blocked in 5% non-fat milk in TBS containing 0.1% Tween 20 (TBS-T) for 1 h at room temperature, followed by overnight incubation at 4°C with primary TPH2 (1:1000, M-59, SantaCruz) and β-actin (1:20000; Sigma) antibodies diluted in TBS-T containing 5% BSA. Membranes were then washed and incubated with peroxidase-conjugated secondary antibodies for 1 h at room temperature. Protein bands were view by a chemiluminescence detection kit (Amersham Biosciences). Band intensities were quantified by densitometric analysis using a BioRad ChemiDoc XRS gel documentation system (BioRad Labs., Hercules, CA, USA), and values were normalized to ß-actin expression.

2.4.3. Radioligand binding experiments

The density of serotonin transporters (SERT) in hippocampal membranes was quantified by measuring the specific binding of 0.1 nM [^{3}H]Paroxetine after incubation with 150 µg protein at 25 °C for 2h in a Tris-HCl buffer (50 mM, pH 7.4), containing 120 mM NaCl and 5 mM KCl to a final volume of 1.6 ml. Clomipramine (100 µM) was used to determine non-specific binding.

The density of dopamine transporter (DAT) in striatal membranes was measured by [^{3}H]WIN 35428 equilibrium binding assays as described elsewhere (Escubedo et al., 2006). Membranes were resuspended in phosphate-buffered 0.32 M sucrose, pH 7.9 at 4 ºC to a concentration of 1 µg/µl. Binding assays were performed in borosilicate glass tubes containing 200 µl of [^{3}H]WIN 35428 dilution in phosphate-buffered 0.32 M sucrose (final radioligand concentration: 5 nM) and 50 µl of membranes. Incubation was done for 2 h at 4 ºC. Non-specific binding was determined in the presence of 30 µM bupropion.

The density of 5-HT$_{2A}$ receptors was measured in membranes of frontal cortex by determining the specific binding of [^{3}H]Ketanserine. These experiments were performed in tubes containing 1 nM [^{3}H]Ketanserin and 100 µg of brain membranes.

Incubation was carried out at 37 °C for 30 min in 50 mM Tris–HCl buffer to a final volume of 0.5 ml. Methysergide (10 μM) was used to determine non-specific binding. All incubations were finished by rapid filtration under vacuum through GF/B glass fibre filters (Whatman, Maidstone, UK). Tubes and filters were washed rapidly 3 times with 4 ml ice-cold buffer and the radioactivity in the filters was measured using a liquid scintillation counter. Specific binding was calculated as the difference between the radioactivities measured in the absence (total binding) and presence (non-specific binding) of an excess of non-labeled ligand. All assays were carried out in duplicate tubes and the mean specific binding for each mouse was calculated.

2.5. Statistical analysis

All data are expressed as mean ± standard error of the mean (S.E.M.). Differences between groups were compared using the appropriate statistical test: Kruskal-Wallis for non- parametric data, followed by U-Mann Whitney and for parametric data two-way ANOVA followed by Tukey post-hoc tests. For qualitative data Chi-square was applied. All calculations were performed using InVivoStat software (British Association of Psychopharmacology).

2.6.Drugs and reagents

Drugs and reagents were obtained from the following sources: aprotinin, bupropion, clomipramine, dexamethasone, imipramine, methysergide, phenylmethylsulfonyl fluoride, sodium orthovanadate were from Sigma-Aldrich (St. Louis, MO, USA). [^{3}H]WIN 35428, [^{3}H]Paroxetine, [^{3}H]Ketanserine were from Perkin-Elmer (Boston, MA, USA). All buffer reagents were of analytical grade.

3. Results

3.1. Forced Swimming Test (FST)

We compared the behavior of SAMR1 (6- to 12-month-old) animals with that of SAMP8 (6- to 12-month-old) animals in the FST. The mice were initially active when placed in the cylinder. After 1-2 min their activity began to decrease and the duration

of immobility was measured. Immobility in the last 3 min of the test is considered an index of despair in response to stress and this behavioral despair is sensitive to antidepressant treatments (Porsolt et al 1978).

The immobility behavior of the two strains differed. Differences were determined for number of immobility events (KW 46,919, d.f. 5, P<0.0001; post-hoc test SAMR1 vs SAMP8 age-matched: $U_{6months}$= 183, P<0.001; $U_{9months}$= 188, P<0.001; $U_{12months}$= 853, P<0.01, Table I) and also for time of immobility in each event (two-way ANOVA, variable strain [F(1,73)=27.9, P<0.001], variable age [F(2,73)=5.4, P<0.01]; Tukey's post-hoc test SAMR1 vs SAMP8 age-matched: 6 months P<0.05, 9 months P<0.001, 12 months P<0.01). SAMR1 animals were observed to constantly change from a mobile to an immobile state so that periods of immobility were short. In contrast, SAMP8 animals remained immobile for longer and consequently the number of times they changed from an active to an inactive state was lower (see Table I). This differential behavior in the FST may indicate that SAMR1 mice make a greater effort to escape from the cylinder than SAMP8 counterparts.

We analyzed the effects of aging on immobility behavior of both strains. . Post-hoc analysis revealed that at an age of 9 months SAMP8 animals spent longer immobility that SAMR1, but returned to a value that did not differ from that of SAMR1 at 12 months, at which it is assumed that both strains are aged (see Figure 1) (variable strain [F(1,73)= 5.24, P<0.05]; variable age x strain [F(2,73)= 4.33, P=0.05]).

Behavior in the FST is reversed by chronic or subacute antidepressant treatment and thus this test is used as an index of depression-like symptomatology [27]. When reversion in depressive behavior was tested using imipramine, we observed that repeated administration of this drug caused a decrease in the total floating period, especially in SAMP8 mice. We measured imipramine reversion by calculating immobility time differences before treatment by imipramine and after 3 or 7 days post-administration. Results indicate that only SAMP8 9 months and 12 months aged were responsive to imipramine (two-way ANOVA, variable strain: 6 months [F(1,30)=1.82, n.s., 9 months [F(1,30)=4.22, P<0.05], 12 months [F(1,72)=4.99, P<0.05] (Figure 2A-C).

Moreover, in order valuate this response, we considered a positive response to imipramine (responsive mice) to be when the reduction in immobility time was equal to or greater than 25%, according previous reports [28, 29]. Therefore, when response to imipramine was tested by means of the FST after 3 days and 7 days of treatment, SAMP8 animals responded to the antidepressant drug, whereas SAMR1 mice were less sensitive to treatment (Figure 2D-F).

3.2 Dexamethasone suppression test

As a measure of the physiological response of the hypothalamic-pituitary-adrenal axis (HPA), we studied the feedback control of cortisol levels after dexamethasone administration. While cortisol levels in 6- and 9-month-old SAMR1 mice decreased after dexamethasone injection, age-matched SAMP8 animals showed no modification in cortisol plasma levels in response to corticoid administration. Post-hoc analysis showed that this difference in HPA functionality was not present in 12-month-old animals of either strain (two-way ANOVA, variable strain [$F(1,59)=7.51$, $P<0.01$], Figure 3).

3.2.Radioligand binding experiments

3.2.1. Serotonin transporter (SERT)

To determine SERT density [^{3}H]Paroxetine binding was measured in the hippocampus and parietal cortex of mice killed at ages of 6, 9 and 12 months.
In hippocampus two-way ANOVA revealed a significant interaction age x strain [$F(2,28) = 3.37$, $P<0.05$]. Post-hoc analysis evidenced that SAMP8 mice hippocampus showed a significantly greater density of [^{3}H]Paroxetine binding sites at 9 and 12 months than age-matched SAMR1 counterparts. These differences were approximately 34% at 9 months and 55% at 12 months compared to SAMR1 mice (see Figure 4A).

Results in the parietal cortex (Figure 4B) showed that aged animals exhibited a decrease in the SERT density (two-way ANOVA, variable age ($F(2,32) = 15.87$, $P<0.001$). Moreover, SAMP8 mice exhibited higher [^{3}H]Paroxetine binding density

than SAMR1 counterparts (two-way ANOVA, variable strain ($F(1,32) = 47.07$, $P<0.001$).

3.2.2. Dopamine transporter

To quantify the density of DAT, [^{3}H]WIN 35428 binding was measured in the striatum of mice killed at 6-, 9- and 12-months of age. ANOVA revealed no statistically significant differences between SAMR1 and SAMP8 animals (see Figure 5). Only 12-month-old SAMR1 mice showed a slight decrease in DA reuptake sites compared with younger animals of the same strain.

3.2.3. 5-HT$_{2A/2C}$ receptors

The density of serotonergic 5-HT$_{2A/2C}$ receptors in the frontal cortex was determined in cortical membranes by measuring the specific binding of [^{3}H]Ketanserine. Although the increase in older animals did not reach statistical significance, the 5-HT$_{2A/2C}$ receptor density tended to increase with age in 12-month-old SAMR1 animals while SAMP8 mice showed constant levels of [^{3}H]Ketanserine binding sites over time (two-way ANOVA, variable age [$F(2,28)=1.77$, $P=0.19$; variable strain [$F(1,28)= 2.67$, $P=0.11$; variable age x strain [$F(2,28)= 1.76$, $P=0.19$] (Figure 6)).

3.3.4 Tryptophan hydroxylase content

Levels of TPH2 were determined by Western blot in the frontal cortex of 6-, 9- and 12-month-old SAMR1 and SAMP8 mice. No significant differences were found in the rate limiting enzyme for 5-HT synthesis (Figure 7).

4. DISCUSSION

In humans, the incidence of depression and impairment of cognitive function, most notably spatial memory, is higher among the elderly; the prevalence of major depression is estimated to be between 1% and 10% of individuals over 60 years of age or older, whereas depressive symptoms may occur in up to 20% (Blazer 1989; Smith 1991; Kumar et al 2006). 5-HT neurotransmission is modified during aging,

which suggests that aging is associated with decreased serotonergic neurotransmission in the CNS Aznar et al 2010; Klein et al 2010). Some of the alterations that reinforce this relationship are the increase in 5-HT terminal autoreceptors, 5-HT_{1B} receptors, and SERT binding sites with age (Duncan et al 2000). Given the importance of threat detection to survival, the neural mechanisms underlying the behavioral and physiological manifestations of anxiety are highly conserved, and thus enable the use of animal models to investigate relationships between stressors and psychopathological function (O'Neill and Moore 2003;).

SAM, a murine model of accelerated senescence, was established by Takeda (1981;1991). Among the SAMP strains, SAMP8 mice show significant impairments in a variety of learning tasks when compared with SAMR1 animals [37]. Further studies suggest that the SAMP8 model exhibits an age-related emotional disorder characterized by reduced anxiety-like behavior [21]. It had been demonstrated an age-dependent diminution in BDNF expression in SAMP8 versus SAMR1 (Alvarez-López et al 2012) and BDNF hypothesis of depression postulates that a reduction in BDNF is directly involved in the pathophysiology of depression, reinforcing the robustness of SAMP8 as a depression-like behavior associated to age [Groves 2007; Kimpton 2012; Lotrich 2012) .

Among the SAMP strains, SAMP10 exhibits age-related brain atrophy and learning impairments in avoidance tasks (Shimada et al 1992;1993). Interestingly, at 7–8 months of age SAMP10 animals show depressive behavior in tail suspension and FST (Onodera et al 2000; Shimada et al 1992), which are common behavioral tests for depression-related behavior in animals (Miyamoto 1997; Buddenberg 2009). An increase in D_2 receptors in the cortex and midbrain, but not in the striatum or cerebellum, was detected in SAMP10 mice (Onodera et al 2000). The same authors observed that this strain showed an increase in cortex and midbrain 5-HT_{1A} receptors with respect to SAMR1, while the binding of ketanserin (5-HT_2) remained unchanged. There are also discrepancies with the changes seen in humans. Miyamoto and co-workers (1986) reported that SAMP10 mice showed behavioral depression compared with the control SAMR1 strain in a tail suspension test, although SAMP8 animals did not. Conversely, in the SAMP8 strain, lower DA replacement is observed in aged animals than in young ones (10 and 2 months) (Ida et al 1985).

Okuma and co-workers (2000) demonstrated changes in neurotransmission by various types of receptors in SAMP8 animals. The noradrenergic system may be

extensively involved in behavioral disorder-related fear, stress and anxiety (Karasawa et al 1999). Biochemical studies performed by Nomura and colleagues demonstrated that there was an age-associated reduction in K^+- or NMDA-evoked release of noradrenalin and an increase in α_2 receptors in the brain of SAMP8 animals (Kitamura et al 1989; Zhao and Nomura 1990). On the basis of these results, it has been suggested that the deterioration in the noradrenergic system contributes to reduced anxiety-like behavior. In contrast, Miyamoto (1997), using the tail suspension test, reported no changes in depression-like behavior in the SAMP8 strain, while SAMP10 mice exhibited significant depression compared with control SAMR1 counterparts.

Here we sought to establish SAMP8 as a behavioral model of age-related depression. For this purpose we first applied the FST. The mice showed depression-like behavior at 9 months of age but not younger, compared with age-matched SAMR1. At 9 months, and given that the lifespan of the SAMP8 strain is 40% shorter than that of SAMR1 mice (9.7 months and 16.3 months, respectively) (Takeda et al 1991), the former were at the end of their lifecycle. Our results indicate that 9-month-old SAMP8 animals show a longer total time of immobility and a lower number of events during immobility than age-matched SAMR1 animals. However, at 12 months of age, there were no significant differences between the two strains. We found a significant increase in responsive SAMP8 mice after imipramine treatment, followed by a reversion of depressive behavior when increases in noradrenalin and 5-HT availability in synapses were induced. These observations provide clues as to the molecular mechanism that induces depressive behavior in these mice.

SAMP8 mice (at 9 and 12 months of age) presented a low availability of 5-HT in the synaptic cleft due to an increase in SERT, measured as [^{3}H]Paroxetine binding, when compared with age-matched SAMR1 mice. The most notable difference in SERT density between the two strains was seen at 9 months of age (25% in hippocampus and 86% in parietal cortex). These results correlate with the high responsiveness to imipramine, a 5-HT reuptake inhibitor, in the behavioral test. Measurements of tryptophan hydroxylase protein levels allowed us to rule out the notion of defective 5-HT synthesis.

The depressive behavior of the SAMP10 strain is related to alterations in brain DA and 5-HT receptors and this mouse has previously been proposed as a useful model of aging associated depressive behavior (Flood et al 1998). Conversely, we found no

significant changes in 5-HT2a receptor densities in SAMR1 or SAMP8, or between ages; although SAMR1 animals showed an age-related up-regulation tendency that was not present in the SAMP8 strain. The involvement of the DA uptake in depression-like behavior observed in SAMP8 mice can be ignored because no changes in DA transporter were detected in SAMR1 or SAMP8 mice at the ages studied.

Depressive behavior is also controlled by the HPA axis. It has been determined that the HPA system is hyperactive in major depressive disorders. Chronic corticosterone treatment of rats and mice produces changes in emotional behavior that may correspond to symptoms of clinical depression and anxiety [for a review, see Gourley and Taylor 2009). The DST has been used extensively to measure the deregulation of the HPA axis in humans (Carrol et al 1981).Concerning the chronic corticosterone model, Iijima et al (2010) showed that the selective serotonin reuptake inhibitor (SSRI) fluvoxamine and the tricyclic antidepressant imipramine are not effective in decreasing the immobility time of chronic corticosterone-treated rats, here we used a single dexamethasone administration as described.

Results on the control of cortisol release by the HPA axis indicate that 6-, and 9-month-old SAMP8 mice were not able to suppress the daily increase in glucocorticoids following dexamethasone administration, and this fact can be considered as a marker of depression-like behavior. The control strain SAMR1 showed a loss in the feedback control of the HPA axis after dexamethasone treatment only at the oldest age tested (12 months), therefore again indicating a relationship between aging and the development of depressive disorders in SAM animals, these occurring both in the senescence accelerated model and in the control strain.

5-HT regulates the activation states of glycogen synthase kinase 3 (GSK3) through 5-HT1 (which inhibits GSK3) and 5-HT2 (which activates the kinase) receptors. Balanced GSK3 activity is essential for 5-HT-regulated brain function and behavior (Polter and Li 2011). Depending on their age, SAMP8 mice are reported to have a higher level of activated GSK3β, which can be demonstrated by hyperphosphorylation of some of its substrates (Canudas et al 2005; Casadesus et al 2012). Moreover, some drugs, such as antidepressants and atypical antipsychotics, regulate GSK3 by inhibiting its activity in the brain [for a review see Polter and Li 2011).

Changes in GSK3β activity in SAMP8 mice reinforce the depression-like behavior reported in the present study, as described in other models (Chen et al 2012).
Our findings demonstrate that 9-month-old SAMP8 mice show age-associated changes in emotional behavior, particularly increasing depression-like behavior. Aged SAMR1 mice (12 months old) also showed a similar depression-like behavior to that of young (6–9-month-old) SAMP8 mice and age matched SAMP8 mice.

5. CONCLUSIONS

Together, our results show that the alterations in depression-like behavior in SAMP8 mice are related to age. Because aging is associated with an increased risk of depression in humans, this mice model can be an useful experimental tool to study age-related mechanism involved in depression. We hypothesize that the age-related increased depression-like behavior in SAMP8 animals is related to dysfunctions in the HPA axis, and to an increase in 5-HT transporter, with slight or marginally significant changes in 5-HT_{2a} receptors. The reversion of this behavior by imipramine treatment, a non-specific inhibitor of noradrenalin and 5-HT transporters, in the FST indicates the strong relation between the depression-like behavior in SAMP8 animals and levels of monoamines. On the basis of our results, we find that the SAMP8 mouse presents an alteration in serotoninergic system, that is relevant because the reduced levels of BDNF in these animals, and lead to develop a depressive-like behavior. These specific changes have been to take in account when research on behavior/learning is performed in this strain as a model of senescence.

6.ACKNOWLEDGEMENTS

We thank the Language Advisory Service of the University of Barcelona for revising the manuscript. This study was supported by grants SAF2010-15948, SAF2011-2363 and SAF2012-39852 from the “Ministerio de Educación y Ciencia”; Plan Nacional sobre Drogas (2010/005); 2009/SGR977 and 2009/SGR00893 from the “Generalitat de Catalunya” and 610RT0405 from the Programa Iberoamericano de Ciencia y Tecnologia para el Desarrollo (CYTED).

7.REFERENCE LIST

Alvarez-López MJ, Castro-Freire M, Cosín-Tomás M, Sanchez-Roige S, Lalanza JF, Del Valle J, Párrizas M, Camins A, Pallás M, Escorihuela RM, Kaliman P. (2013) Long-term exercise modulates hippocampal gene expression in senescent female mice. J Alzheimers Dis. 33(4):1177-90. doi: 10.3233/JAD-121264.

Arango V, Ernsberger P, Marzuk PM, Chen JS, Tierney H, Stanley M, Reis DJ, Mann JJ (1990). Autoradiographic demonstration of increased serotonin 5-HT2 and beta-adrenergic receptor binding sites in the brain of suicide victims. Arch Gen Psychiatry 47(11):1038-1047.

Aznar S, Klein AB, Santini MA, Knudsen, GM, Henn F, Gass P, Vollmayr B (2010) Aging and depression vulnerability interaction results in decreased serotonin innervation associated with reduced BDNF levels in hippocampus of rats bred for learned helplessness. Synapse 64(7):561-565.

Blazer D (1989) Depression in the elderly. N. Eng. J. Med. 320:164-166.

Blier P, de Montigny C (1994). Current advances and trends in the treatment of depression. Trends Pharmacol Sci. 15(7): 220-226.

Bourin M, Baker GB (1996) The future of antidepressants. Biomed Pharmacother 50(1):7-12.

Buddenberg TE, Komorowski M, Ruocco LA, Silva MA, Topic B (2009) Attenuating effects of testosterone on depressive-like behavior in the forced swim test in healthy male rats. Brain Res. Bull. 79:182–186.

Butterfield DA, Poon HF (2005) The senescence-accelerated prone mouse (SAMP8): a model of age-related cognitive decline with relevance to alterations of the gene expression and protein abnormalities in Alzheimer's disease. Exp Gerontol 40(10):774-783.

Canudas AM, Gutierrez-Cuesta J, Rodríguez MI, Acuña-Castroviejo, D., Sureda, F.X., Camins, A., Pallàs, M. (2005). Hyperphosphorylation of microtubule-associated protein tau in senescence-accelerated mouse (SAM). Mech. Ageing Dev. 126(12):, 1300-1304.

Carrol BJ, Feiberg M, Greden JF, Tarika J, Albala AA, Haskett RF, James NM, Kronfol Z, Lohr, N., Steine, M., de Vigne, J.P., Young, E. (11981). A specific laboratory test for the diagnosis of melancholia. Standarization, validation and clinical utility. Arch. Gen. Psychiatry 38:15-22.

Casadesús G, Gutierrez-Cuesta J, Lee HG, Jiménez A, Tajes M, Ortuño-Sahagún D, Camins A, Smith MA, Pallàs M (2012) Neuronal Cell Cycle Re-Entry Markers are Altered in the Senescence Accelerated Mouse P8 (SAMP8). J Alzheimers Dis 30(3):573-583

Chen WQ, Ma H, Bian JM, Zhang YZ, Li J (2012) Hyper-phosphorylation of GSK-3β: Possible roles in chlorpyrifos-induced behavioral alterations in animal model of depression. Neurosci. Lett. 0304-3940(12)01178-0. doi: 10.1016/j.neulet.2012.08.084.

Duncan, MJ, Crafton CJ, Wheeler DL (2000) Aging regulates 5-HT(1B) receptors and serotonin reuptake sites in the SCN. Brain Res 856(1-2): 213-219.

Flood JF, Farr SA, Uezu K, Morley JE (1998) Age-related changes in septal serotonergic, GABAergic and glutamatergic facilitation of retention in SAMP8 mice. Mech Ageing Dev 105(1-2):173-188.

Gareri P, De Fazio P, De Sarro G (2002) Neuropharmacology of depression in aging and age-related diseases. Ageing Res Rev 1(1):113-134.

Gourley SL, Taylor JR (2009). Recapitulation and reversal of a persistent depression-like syndrome in rodents. Curr Protoc Neurosci.Chapter 9:Unit 9.32.

Groves JO (2007) Is it time to reassess the BDNF hypothesis of depression? Mol Psychiatry. 12(12):1079-88.

Gutierrez-Cuesta J, Tajes M, Jimenez A, Coto-Montes A, Camins A and Pallàs M (2008) Evaluation of potential pro-survival pathways regulated by melatonin in a murine senescence Model. Journal of Pineal Research 45:497–505.

Ida Y, Tanaka M, Tsuda A, Tsujimaru S, Nagasaki, N (1985) Attenuating effect of diazepam on stress-induced increases in noradrenalin turnover in specific brain regions of rats. Antagonism by Ro 15-1788. Life Sci 37:2491–2498.

Iijima M, Ito A, Kurosu S, Chaki S (2010) Pharmacological characterization of repeated corticosterone injection-induced depression model in rats. Brain Res 1359:75e80.

Karasawa N, Yamawaki Y, Nagatsu T, Kawase T, Nishiyama K, Watanabe K, Onozuka M, Nagatsu I (1999) Age-associated changes in the dopamine synthesis as determined by GTP cyclohydrolase I inhibitor in the brain of senescence-accelerated mouse-prone inbred strains (SAMP8). Neurosci. Res. 35(1):31-36.

Kimpton J (2012) The brain derived neurotrophic factor and influences of stress in depression. Psychiatr Danub. Suppl 1:S169-171.

Kitamura Y, Zhao XH, Ohnuki T, Takei M, Nomura Y (1989) Ligand-binding characteristics of [3H]QNB, [3H]prazocin, [3H]rauwolsine, [3H]TCP and [3H]nitrendipine to cerebral cortical and hippocampal membranes of senescence-accelerated mouse. Neurosci Lett 196:334–338.

Klein AB, Santini MA, Aznar S, Knudsen GM, Rios M (2010) Changes in 5-HT2A-mediated behavior and 5-HT2A- and 5-HT1A receptor binding and expression in conditional brain-derived neurotrophic factor knock-out mice. Neuroscience 169(3):1007-1016.

Kumar R, Jorm AF, Parslow RA, Sachdev PS (2006) Depression in mild cognitive impairment in a community sample of individuals 60-64 years old. Int Psychogeriatr 18(3):471-480.

Lotrich F (2012) Inflammatory cytokines, growth factors, and depression. Curr Pharm Des 18(36):5920-35.

Mann JJ, Stanley M, McBride PA, McEwen BS (1986) Increased serotonin2 and beta-adrenergic receptor binding in the frontal cortices of suicide victims. Arch Gen Psychiatry 43(10):954-959.

Miyamoto M (1997) Characteristics of age-related behavioral changes in senescence accelerated mouse SAM-P8 and SAM-P10. Exp Gerontol 32:139–148.

Miyamoto M, Kiyota Y, Nishiyama M, Nagaoka A (1992) Senescence-accelerated mouse (SAM), age-related reduced anxiety-like behavior in the SAM-P/8 strain. Physiol Behav 51(5):979-985.

Miyamoto M, Kiyota Y, Yamazaki N, Nagaoka A, Matsuo T, Nagawa Y, Takeda T (1986) Age-related changes in learning and memory in the senescence-accelerated mouse (SAM). Physiol Behav 38(3):399-406.

Murray CJ, Lopez AD (1997) Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. Lancet 349(9063):1436-1442.

Nutt DJ (2006) The role of dopamine and norepinephrine in depression and antidepressant treatment. J Clin Psychiatry. 67 Suppl 6:3-8.

O'Neil MF, Moore NA (2003) Animal models of depression: are there any? Hum. Psychopharmacol Clin Exp 18:239–254

Okuma Y, Murayama T, Tha KK, Yamada C, Hosokawa M, Ishikawa A, Watanabe R, Maekawa M, Nomura Y (2000) Learning deficiency and alterations

in acetylcholine receptors and protein kinase C in the brain of senescence-accelerated mouse (SAM)-P10. Mech Ageing Dev 114(3):191-199.

Onodera T, Watanabe R, Tha KK, Hayashi Y, Murayama T, Okuma Y, Ono C, Oketani Y, Hosokawa, M., Nomura, Y. (2000). Depressive behavior and alterations in receptors for dopamine and 5-hydroxytryptamine in the brain of the senescence-accelerated mouse (SAM)- P10. Jpn J Pharmacol 83:312–318.

Owens MJ, Nemeroff CB (1998). The serotonin transporter and depression. Depress Anxiety. 8 Suppl 1:5-12.

Ownby RL, Crocco E, Acevedo A, John V, Loewenstein D (2006) Depression and risk for Alzheimer disease, systematic review, meta-analysis, and metaregression analysis. Arch Gen Psychiatry 63:530–538.

Peroutka SJ, Snyder SH (1980). Long-term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding. Science 210(4465):88-90.

Petursdottir A L, Farr SA, Morley JE, Banks WA, Skuladottir GW (2007) Lipid peroxidation in brain during aging in the senescence-acceleratedmouse (SAM). Neurobiol Aging 28:1170–1178.

Polter AM, Li X. (2011) Glycogen Synthase Kinase-3 is an Intermediate Modulator of Serotonin Neurotransmission. Front Mol Neurosci 4:31.

Porsolt RD, Anton G, Blavet N, Jalfre M (1978) Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 47(4):379-391.

Porsolt RD (2000). Animal models of depression: utility for transgenic research, Rev Neurosci 11:53–58.

Porsolt RD, Le Pichon M, Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. Nature 266:730–732.

Potter GG, Wagner HR, Burke JR, Plassman BL, Welsh-Bohmer KA, Steffens DC (2012) Neuropsychological Predictors of Dementia in Late-Life Major Depressive Disorder. Am J Geriatr Psychiatry. doi: 10.1097/JGP.0b013e318248764e.

Shimada A, Ohta A, Akiguchi I, Takeda T (1992) Inbred SAM-P/10 as a mouse model of spontaneous, inherited brain atrophy. J Neuropathol Exp Neurol 51:440–450.

Shimada A, Ohta A, Akiguchi I, Takeda T (1993) Age-related deterioration in conditional avoidance task in the SAM-P/10 mouse, an animal model of spontaneous brain atrophy. Brain Res. 608:266–272.

Smith GW (1991) Recognition and treatment of depression in the elderly. J Clin Psychiatry 52:111-22.

Stockmeier CA (1997) Neurobiology of serotonin in depression and suicide. Ann N Y Acad Sci 836:220-232.

Takeda T (2009) Senescence-accelerated mouse (SAM) with special references to neurodegeneration models, SAMP8 and SAMP10 mice. Neurochem Res 34:4):639-659.

Takeda T, Hosokawa M, Higuchi K (1991) Senescence-accelerated mouse (SAM): a novel murine model of accelerated senescence. J Am Geriatr Soc 39(9):911-919.

Takeda T, Hosokawa M, Takeshita S, Irino M, Higuchi K, Matsushita T, Tomita Y, Yasuhira K, Hamamoto H, Shimizu K, Ishii M, Yamamuro T (1981) A new murine model of accelerated senescence. Mech. Ageing Dev 17(2):183-194.

Waider J, Araragi N, Gutknecht L, Lesch KP (2011) Tryptophan hydroxylase-2 (TPH2) in disorders of cognitive control and emotion regulation: a perspective. Psychoneuroendocrinology 36:393-405.

Yamada J, Sugimoto Y (2001) Effects of 5-HT(2) receptor antagonists on the anti-immobility effects of imipramine in the forced swimming test with mice. Eur J Pharmacol 427(3):221-225.

Yates M, Leake A, Candy JM, Fairbairn AF, McKeith IG, Ferrier IN (1990). 5HT2 receptor changes in major depression. Biol. Psychiatry. 27(5):489-496.

Zhao,XH, Nomura Y (1990) Age-related changes in uptake and release on L-[3H]noradrenaline in brain slices of senescence accelerated mouse. Int J Dev 8(3):267-272.

Legends for figures

Figure 1: Differences in the total immobility time in seconds. Bars represent mean ± SEM from 9 to 14 animals. Two-way ANOVA and Tukey's post-hoc test .$^{**}P$<0.01 SAMP8 *vs.* age-matched SAMR1.

Figure 2: Differences in the total immobility time in seconds before and after 3- and 7days of imipramine treatment at 6- (A), 9-(B) and 12-month-old (C) SAMR1 and SAMP8 (two-way ANOVA, variable strain: 6 months [F(1,30)=1.82, n.s.], 9 months [F(1,30)=4.22, P<0.05], 12 months [F(1,72)=4.99, P<0.05]). Right column: Percentage of responsive mice after 3 or 7 days of imipramine treatment in animals aged 6 months (D), 9 months (E) and 12 months (F). We considered a positive response to imipramine (responsive mice) to be when the reduction in immobility time was equal to or greater than 25%. Exact Fisher test:* P<0.05, ***P<0.001 SAMP8 vs. age-matched SAMR1.

Figure 3: Feedback control of corticosterone levels before and after dexamethasone administration in 6-, 9- and 12-month-old SAMR1 and SAMP8 mice. Bars represent mean ± SEM from 9 to 10 animals; experiments were carried out in duplicate. Two-way ANOVA and Tukey's post-hoc test: $^{**}P$<0.01 and $^{***}P$<0.001 SAMP8 *vs.* age-matched SAMR1.

Figure 4: [^{3}H]Paroxetine binding indicative of serotonin reuptake sites in the hippocampus (A) and in the parietal cortex (B) of 6-, 9- and 12-month-old SAMR1 and SAMP8 mice. Bars represent mean ± SEM from 6 different samples carried out in triplicate. Two-way ANOVA and Tukey's post-hoc test: $^{***}P$<0.001 *vs.* 6-month-old SAMR1. $^{\#}P$<0.05, $^{\#\#}P$<0.01 and $^{\#\#\#}P$<0.001 *vs.* 6-month-old SAMP8. $^{\Phi}P$<0.05 and $^{\Phi\Phi}P$<0.01 SAMP8 *vs.* age-matched SAMR1.

Figure 5: [^{3}H]WIN 35428 binding indicative of DA reuptake sites in the striatum of 6-, 9- and 12-month-old SAMR1 and SAMP8 mice. Bars represent mean ± SEM from 6 different samples carried out in triplicate. ANOVA revealed a non-significant differences.

Figure 6: [^{3}H]Ketanserine binding indicative of 5-$HT_{2A/2C}$ receptor levels in the frontal cortex of 6-, 9- and 12-month-old SAMR1 and SAMP8 mice. Bars represent mean ± SEM from 6 different samples carried out in triplicate. ANOVA revealed a non-significant effect of age on receptor binding but also a non-significant difference between SAMP8 and SAMR1 at all ages.

Figure 7: Panel A: Representative Western blots showing individual tryptophan hydroxylase 2 expression in 12-month-old mouse striatum**.** Panel B**:** shows quantification of tryptophane hydroxylase 2 (TPH2) levels from all the Western blots performed. β-actin expression was used as a gel load control and to normalize the results as the ratio TPH/β-actin expression. Western blots were performed with samples from at least four animals from each group in duplicate. ANOVA revealed a non-significant differences in expression levels of TPH/β-actin but also a non-significant difference between SAMP8 and SAMR1 at all ages.

Figure 1
Click here to download Figure: Figure 1.doc

Figure 1,Pérez-Cáceres et al.

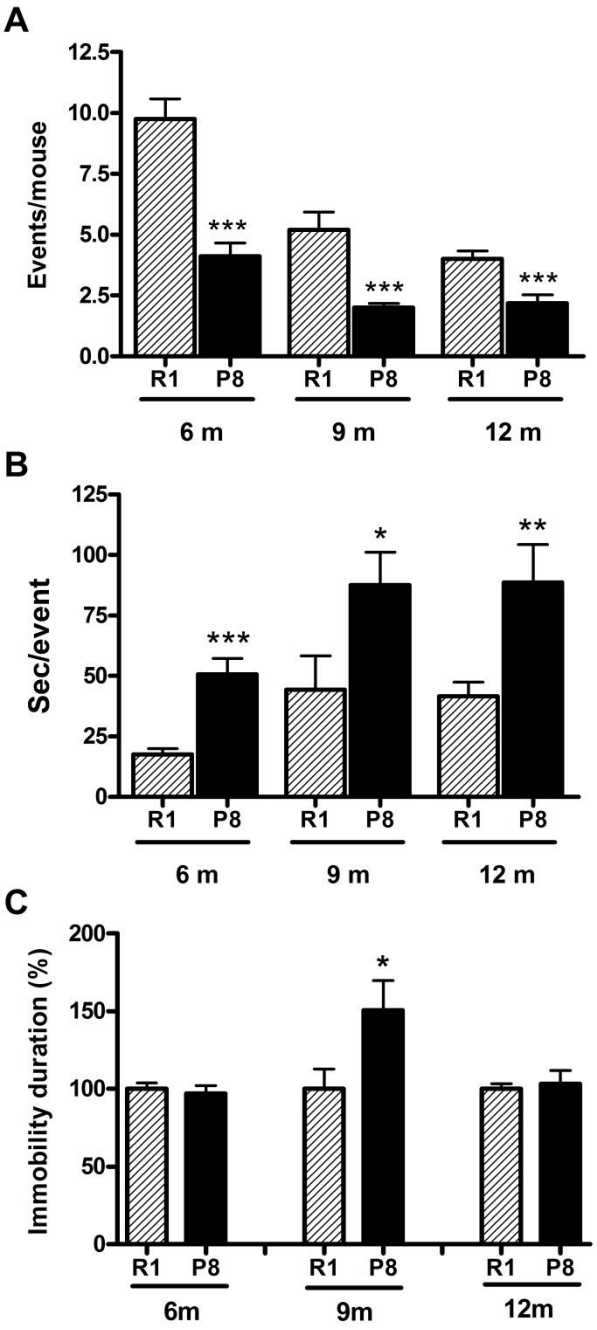
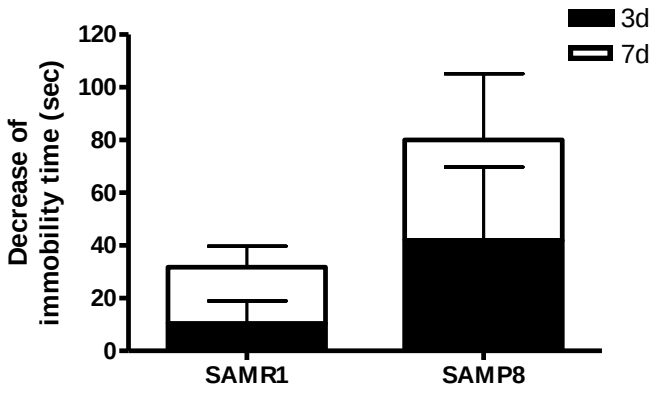


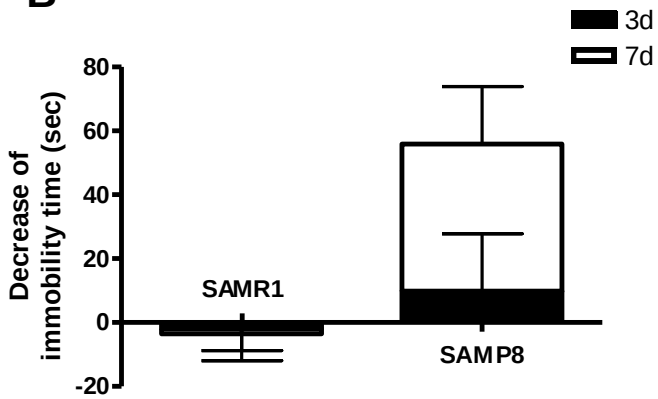
Figure 2
Click here to download Figure: Figure 2.doc

A



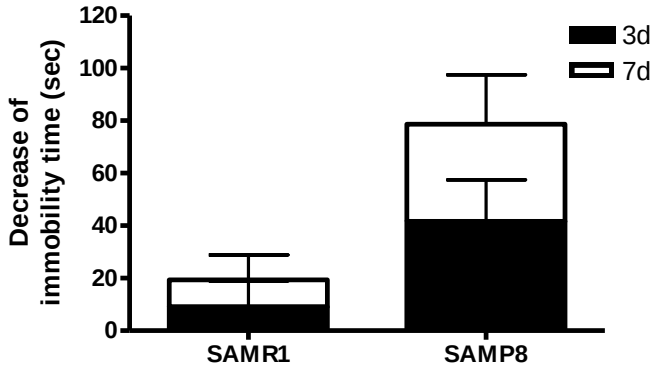
D	STRAIN	Percentage of responsive mice
SAMR1	*3 days*	10
	7 days	30
SAMP8	*3 days*	55.6*
	7 days	50*

B



E	STRAIN	Percentage of responsive mice
SAMR1	*3 days*	0
	7 days	25
SAMP8	*3 days*	22.2
	7 days	55.6

C



F	STRAIN	Percentage of responsive mice
SAMR1	*3 days*	7.4
	7 days	7.4
SAMP8	*3 days*	54.5***
	7 days	45.5***

Figure 3
Click here to download Figure: Figure 3.doc

Figure 3, Pérez-Cáceres et al.

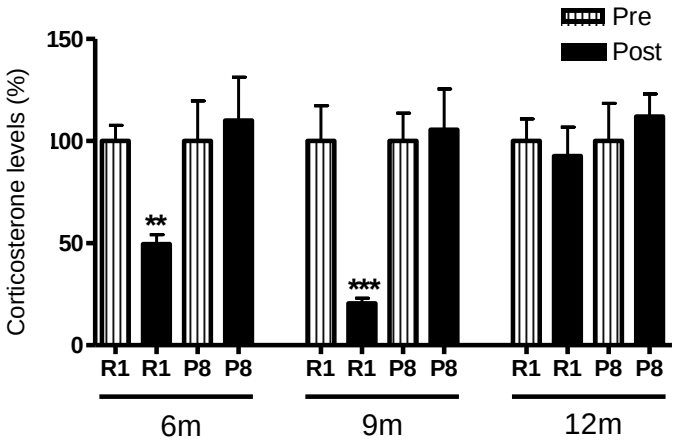
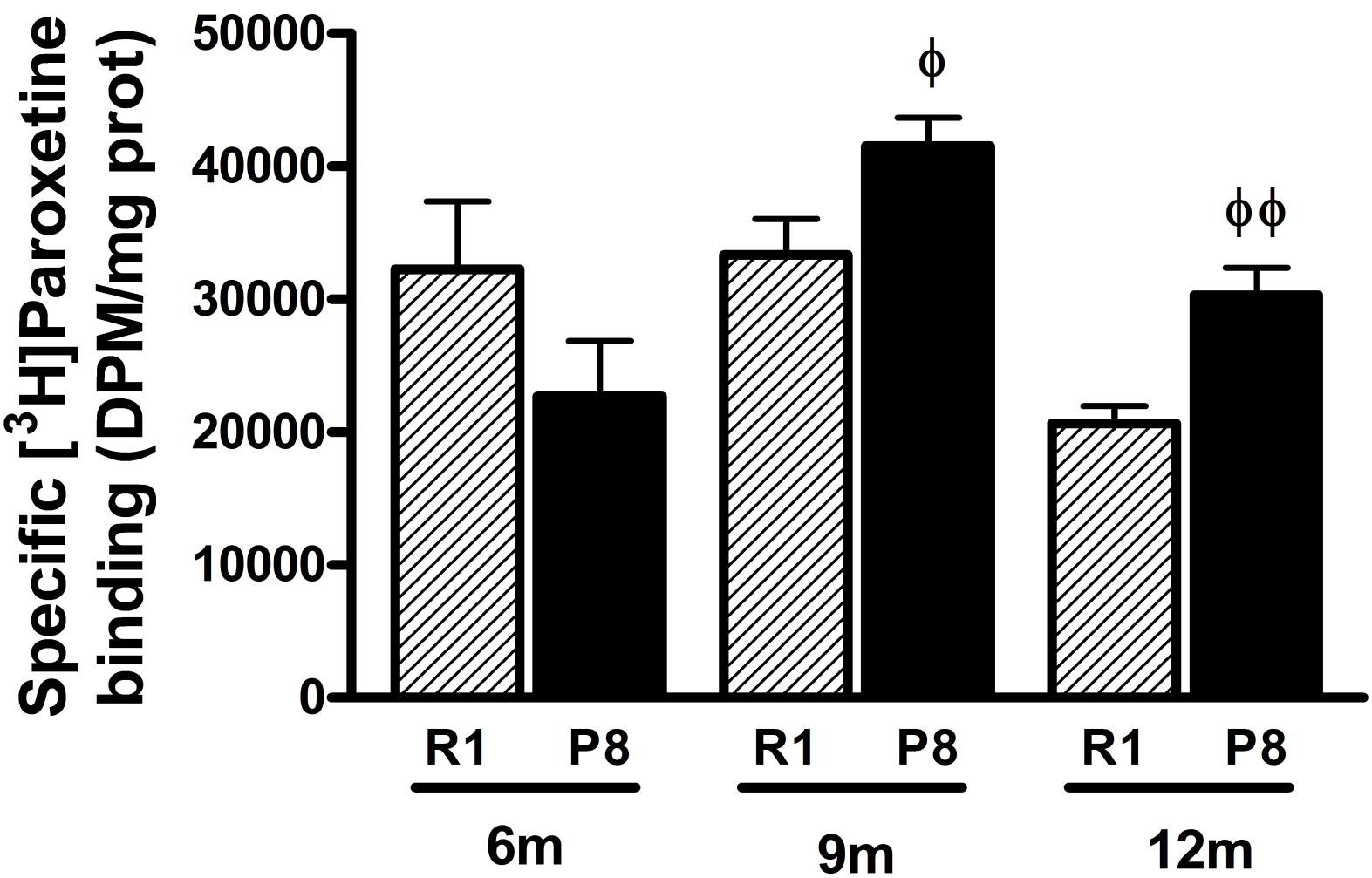


Figure 4

Click here to download Figure: Fig 4.pdf

A



B

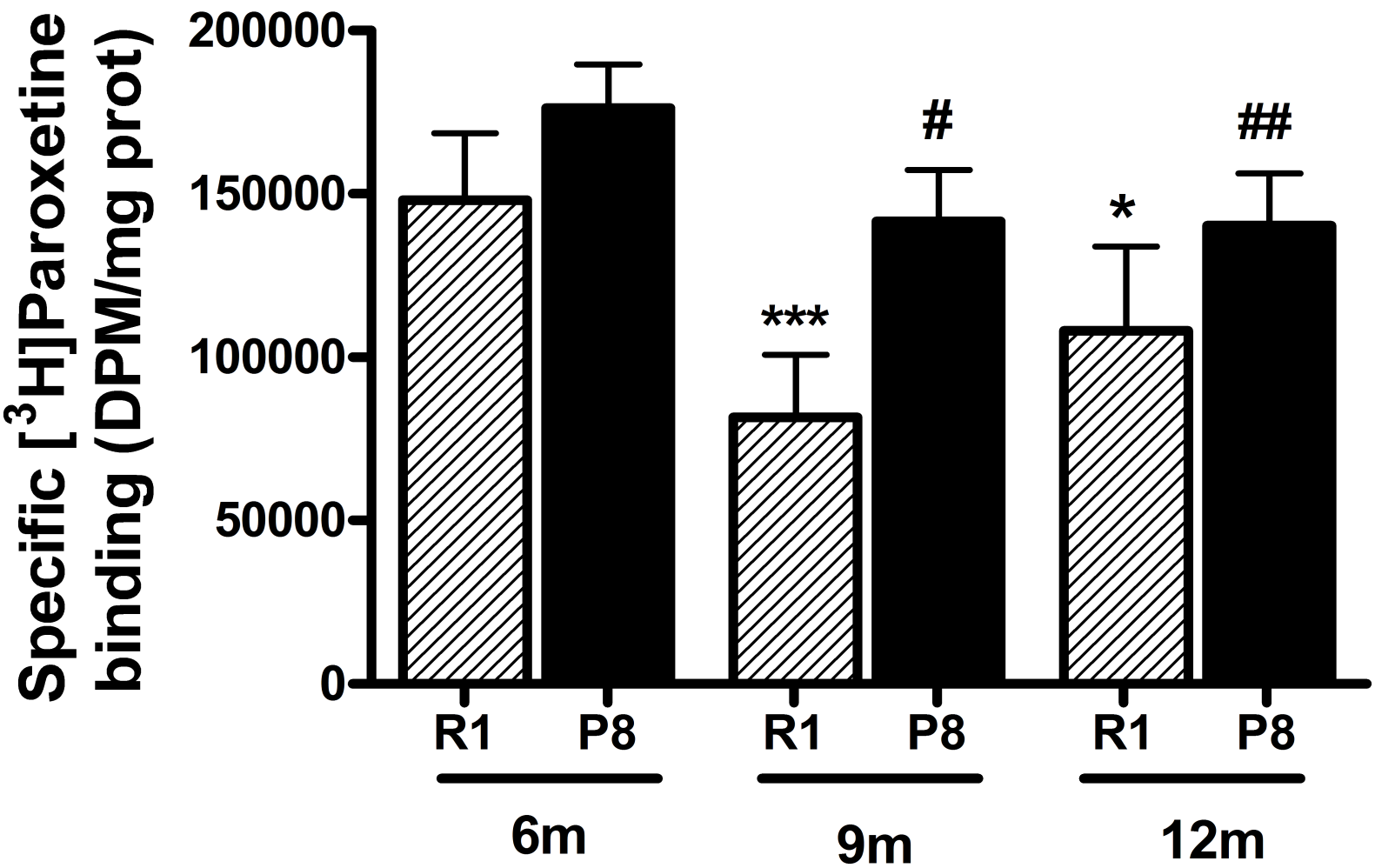


Figure 5
Click here to download Figure: Fig 5.pdf

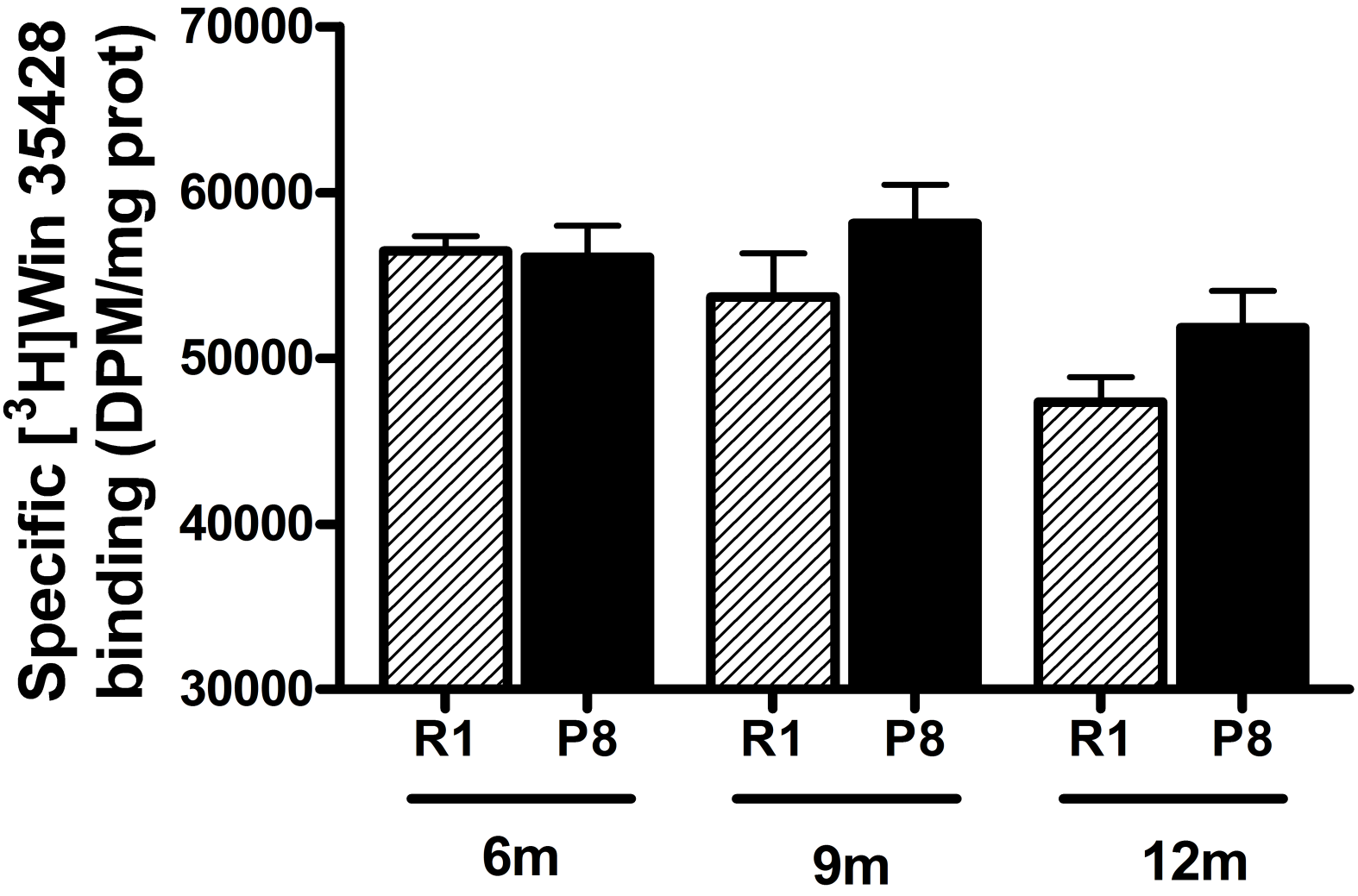


Figure 6
Click here to download Figure: Fig 6.pdf

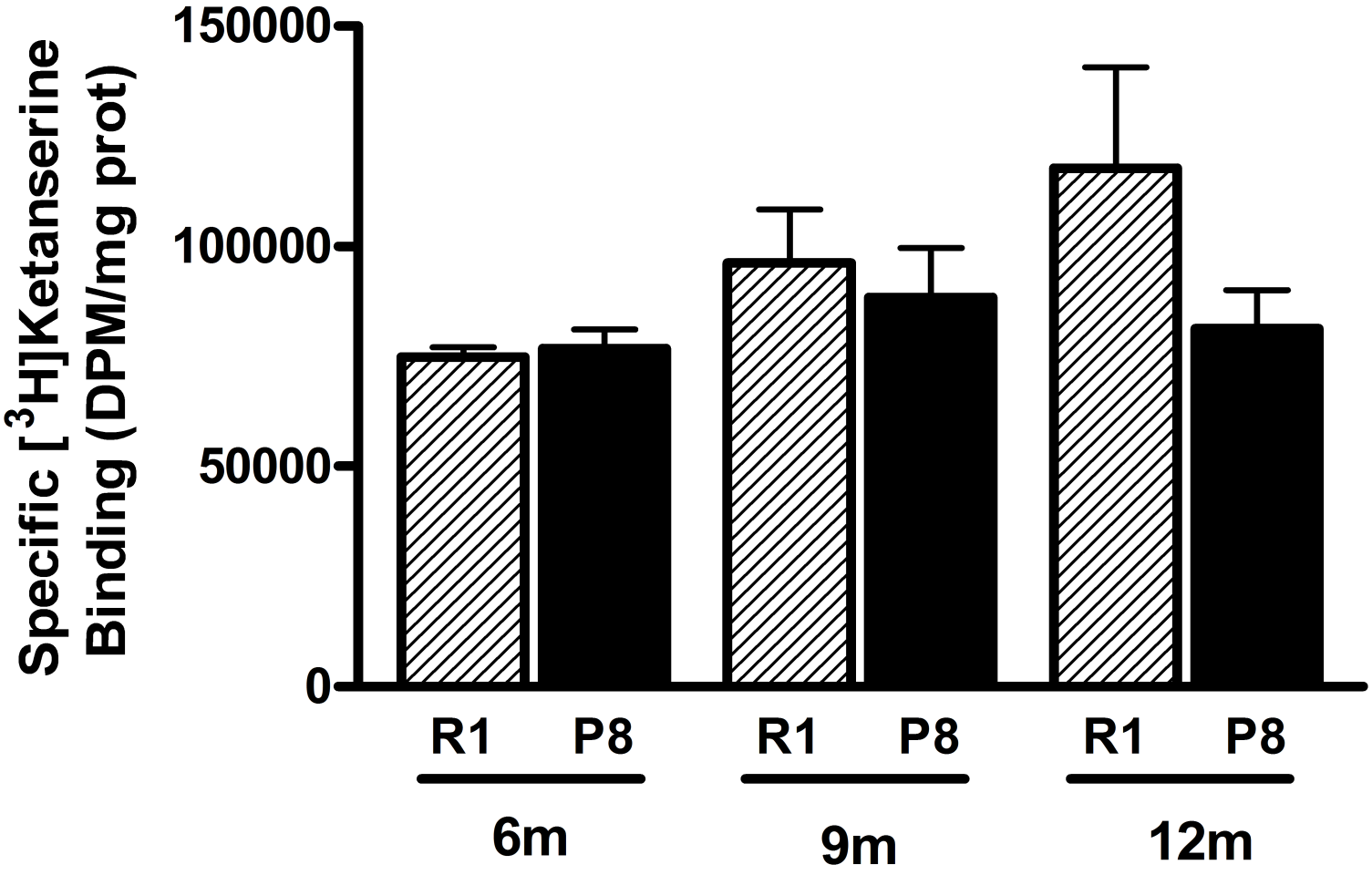


Figure 7
Click here to download Figure: Figure 7PECA.doc

Figure 7, Pérez-Cáceres et al.