ELSEVIER

Brain Research 882 (2000) 149–154

BRAIN RESEARCH

www.elsevier.com/locate/bres

Research report

Regional differences in brain monoamine oxidase subtypes in an animal model of geriatric depression: effects of olfactory bulbectomy in young versus aged rats

Theodore A. Slotkin[a,*], Frederic J. Seidler[a], James C. Ritchie[b]

[a]*Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710, USA*
[b]*Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA 30322, USA*

Accepted 15 August 2000

Abstract

Geriatric depression is often associated dysregulation of the hypothalamus–pituitary–adrenal (HPA) axis, and with poor responsiveness to antidepressants that work through inhibition of monoamine reuptake; accordingly, it has been suggested that MAO inhibitors may represent a therapeutic alternative in this group. In the current study, we evaluated expression of MAO subtypes in brain regions of young and aged rats subjected to olfactory bulbectomy (OBX), a procedure that reproduces many of the biochemical and functional changes associated with human depression. Activities of both MAO A and B were elevated in aged rats as compared to young rats in most regions, but not in the midbrain, and the OBX lesion failed to produce any change in this pattern. These results stand in contrast to the differential effects of glucocorticoids, which reduce brain MAO in young animals but induce activity in aged rats. Our results support the view that the aged brain possesses biochemical characteristics that distinguish its monoamine biochemistry from that of young brain, and that these distinctions may work in conjunction with HPA axis dysregulation to influence the etiology and therapy of geriatric depression. The use of appropriate animal models for depression and for disruption of HPA axis function can allow for the testing of potential human biomarkers (such as platelet MAO) that may serve to predict treatment outcome.

Theme: Disorders of the nervous system

Topic: Neuropsychiatric disorders

Keywords: Aging; Depression; Hypothalamus–pituitary–adrenal axis; Monoamine oxidase; Olfactory bulbectomy

1. Introduction

In geriatric populations, the biological abnormalities associated with depression, and the corresponding efficacy of standard treatments, appear to differ substantially from those in younger patients. In particular, monoamine reuptake inhibitors have a higher rate of failure in the elderly [1,5,7,8,17,24]; we found that the platelet serotonin transporter, a surrogate marker for serotonin transport in the central nervous system, is resistant to inhibition of serotonin uptake by antidepressants in elderly depressed patients [31]. Accordingly, it has been suggested that MAO inhibitors may prove more useful in geriatric depression [16]. Many monoamine reuptake inhibitors are also anticholinergic agents and thus worsen cognitive impairment in the elderly, an effect not shared by MAO inhibitors [19,25]. It then becomes vital to understand how aging and depression, separately and together, influence brain MAO activity, in order to optimize the therapeutic use of these drugs.

One of the most useful animal models for human depression is the olfactory bulbectomized (OBX) rat, which exhibits behavioral and biochemical characteristics that, as in man, are reversed after chronic, but not acute, antidepressant therapy (reviews [10,12]). Importantly for our studies, these animals exhibit abnormalities of monoaminergic function that parallel the putative changes in

Abbreviations: ANOVA, analysis of variance; HPA, hypothalamus–pituitary–adrenal; MAO, monoamine oxidase; OBX, olfactory bulbectomy

*Corresponding author. Tel.: +1-919-681-8015; fax: +1-919-684-8197.

E-mail address: t.slotkin@duke.edu (T.A. Slotkin).

0006-8993/00/$ – see front matter
PII: S0006-8993(00)02859-6

human depression [35]. In a previous study, we found that, in aged rats, OBX lesions produce changes in serotonin transporter expression and in synaptic reactivity to serotonin that are distinct, and often opposite, from the effects of lesioning in younger animals [29]. In the current study, we have extended this approach to the measurement of MAO subtypes in brain regions of young and aged OBX rats. We selected animals at 20 months of age rather than examining the extreme of the life span; neurodegeneration, synaptic dysmorphology and neuronal loss are likely to be present when very old rats are used, obscuring or exacerbating any primary effects on cellular function [14].

2. Materials and methods

2.1. Animal treatments

Studies were carried out in accordance with the declaration of Helsinki and with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health. Male Sprague–Dawley rats (Camm Research Institute, Wayne, NJ, USA) were obtained at 9 weeks or 19 months old and were housed individually with free access to food and water and a 12 h light–dark cycle (0600–1800). Animals were handled and weighed daily from the time of arrival until completion of the study. One week after arrival, animals were anesthetized with 6.5 mg/kg of xylazine and 44 mg/kg of ketamine, given intraperitoneally. The top of the skull was shaved and swabbed with an antiseptic, after which a midline frontal incision was made in the scalp and the skin was retracted bilaterally. Burr holes (2–3 mm) were drilled into the skull 2 mm lateral to the bregma suture, after which the olfactory bulbs were severed from the frontal cortex and aspirated according to established protocols [10,12]. The cavity was packed with Surgicel®, the skin was closed with surgical clips and bupivacaine was applied. The animals were given 40 000 IU/kg of procaine penicillin intramuscularly, and were allowed to recover with warming to maintain body temperature. Sham-operated animals underwent the same procedure except for excision and aspiration of the olfactory bulbs.

Experiments were carried out 25 days after surgery. Animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.), decapitated and the brain was dissected to obtain the frontal/parietal cortex, hippocampus, midbrain and cerebellum (including flocculi). In the sham-operated animals, care was taken to exclude the olfactory bulbs from the dissection. Brain regions were frozen in liquid nitrogen and maintained at −45°C until used.

2.2. MAO activity

The activities of the two MAO subtypes were assessed by a modification of standard techniques [15,22]. Tissues were homogenized in ten volumes of 0.1 M phosphate buffer (pH 7.2). Duplicate assays (110 μl total volume) were prepared with homogenate (or boiled tissue for blanks) corresponding to 5 mg of tissue with or without clorgyline (1 μM) to inhibit MAO A or L-deprenyl (10 μM) to inhibit MAO B (both compounds obtained from Research Biochemicals International, Natick, MA, USA). Samples were incubated for 15 min at 37°C, after which 120 nmol of benzylamine[7-^{14}C] (ICN, Irvine, CA, USA) were added in 250 μl of Tris buffer (pH 9.1). Thirty min later, the reaction was stopped with 50 μl of 6 N HCl and the samples were extracted into hexane, after which the reaction products were counted. A standard sample of platelet-rich plasma was run with every set of assays to ensure reproducibility.

2.3. Data analysis

Results are presented as means and standard errors. Age- or treatment-related differences were analyzed first by a global ANOVA incorporating all variables: age, surgical treatment, brain region, and MAO subtype; data were log-transformed because of heterogeneous variance and the subtype was considered to be a repeated measure, since both type A and B activities were measured in the same tissue sample. Where this initial test indicated an interaction between variables, appropriate lower-order ANOVAs were carried out to identify significant main effects of each variable and further interactions. Post-hoc tests of individual differences were evaluated with Fisher's Protected Least Significant Difference only where significant interactions were found for age×treatment, or for age×treatment×other variables; in the absence of interaction terms, only main effects are reported. Significance was assumed at $P<0.05$ for main effects and at $P<0.1$ for interactions [32].

3. Results

Twenty-five days after sham surgery or OBX lesioning, body weights of the lesioned animals were indistinguishable from the sham group in both young and aged rats (Table 1). Nevertheless, there were significant, regionally-selective effects on brain region weights. Both young and aged rats showed OBX-induced reductions in weight in the frontal/parietal cortex, with a significantly larger proportional loss in the aged animals (OBX×age interaction). A similar age-dependent effect was seen in a region more distal to the lesion, the midbrain; in young animals, there was no significant deficit caused by OBX, whereas a robust effect was seen in the aged OBX group. In contrast, no effects were seen for tissue weight in either hippocampus or cerebellum for either young or aged rats.

Global statistical analysis of the MAO data indicated main effects of age, brain region, and MAO subtype, as

Table 1
Body and brain region weights[a]

	Young sham	Young OBX	Aged sham	Aged OBX	ANOVA
	(11)	(17)	(6)	(6)	
Body (g)	391±13	383±6	645±13	632±14	NS
Frontal/parietal cortex (mg)	597±17	526±16[b]	668±48	492±28[b]	Sham >OBX, $P<0.0001$; OBX×age, $P<0.07$
Hippocampus (mg)	127±4	122±3	152±6	151±5	NS
Midbrain (mg)	314±5	310±4	379±16	334±14[b]	Sham >OBX, $P<0.01$; OBX×age, $P<0.03$
Cerebellum (mg)	296±8	292±4	331±9	319±15	NS

[a] Data represent means and standard errors obtained from the number of animals in parentheses. ANOVA for each region appears in the right column. Across all regions, ANOVA indicates a significant reduction caused by OBX ($P<0.0008$) and significant interactions of OBX×region ($P<0.0001$) and OBX×region×age ($P<0.05$).
[b] Denotes values for which the OBX group differs from the corresponding sham-operated controls.

well as interactions among these three variables (Table 2). Notably, there was neither a main effect of OBX lesioning, nor did the lesioning treatment interact with any of the other variables. Across all four regions, MAO B predominated over MAO A. OBX lesioning did not alter the overall pattern of preponderance of MAO B over MAO A, nor did it differentially affect subtype predominance in aged versus young animals (no interactions of OBX× subtype, OBX×age, or OBX×subtype×age). Separate examination of each MAO subtype gave essentially the same results: main effects and interactions for age and region, but no effect of lesioning. Accordingly, we examined the comparative effects of aging, with and without OBX lesioning, separately in each brain region (Fig. 1). In the frontal/parietal cortex, aged rats showed higher MAO B activities than young rats but OBX lesioning had no significant effect on either subtype. The hippocampus and cerebellum showed higher activities of both MAO subtypes in the aged brain, again without any significant effect of OBX lesioning in either age group. In contrast, the midbrain did not show any differences in MAO A or B between young and aged rats, and as in the other regions, OBX had no effect.

Table 2
Statistical analyses of MAO subtypes[a]

	Both subtypes	MAO A	MAO B
Age	$P<0.006$	NS	$P<0.0002$
Treatment	NS	NS	NS
Region	$P<0.03$	$P<0.0001$	$P<0.0002$
Subtype	$P<0.0001$	–	–
Age×treatment	NS	NS	NS
Age×region	$P<0.03$	$P<0.03$	$P<0.06$
Age×subtype	NS	–	–
Treatment×region	NS	NS	NS
Treatment×subtype	NS	–	–
Region×subtype	$P<0.0001$	–	–

[a] In addition to the main effects and interactions shown above, three- and four-way interactions that failed to show significant differences were: age×treatment×region, age×treatment×subtype, age×region×subtype, treatment×region×subtype, and age×treatment×region×subtype.

4. Discussion

In the present study, we found higher MAO activities for both enzyme subtypes in hippocampus and cerebellum of aged rats and higher MAO B activities in aged frontal/parietal cortex; overall, MAO B activity was greater than MAO A activity. These results for the aged brain are all in keeping with earlier work [2,30,33,37]; however there was no age-related difference in the midbrain, indicating that the increased expression of MAO that occurs with aging is actually regionally-selective, rather than representing a global change in all parts of the brain. Similarly, the fact that one region (frontal/parietal cortex) showed greater aging-related effects on MAO B than MAO A, differences that were not seen in hippocampus or cerebellum, indicates that expression of individual subtypes is regulated independently within each brain region. Notably, we found no change after OBX lesions, regardless of whether the surgery was conducted in young or aged rats. In contrast to this finding, earlier work with chronic glucocorticoid administration identified clear-cut differences in MAO regulation between young and aged rats, with reductions in young animals [6,36] but increases in aged rats, selectively greater for MAO A [30]. Taken together these results indicate that hypothalamus–pituitary–adrenal (HPA) axis function plays a larger role in MAO subtype expression than does the alteration in monoamine pathways elicited by the OBX model of depression. This is particularly important in light of the connection between HPA axis dysfunction and human depression. Elderly depressives show an unusually high incidence of HPA dysregulation as

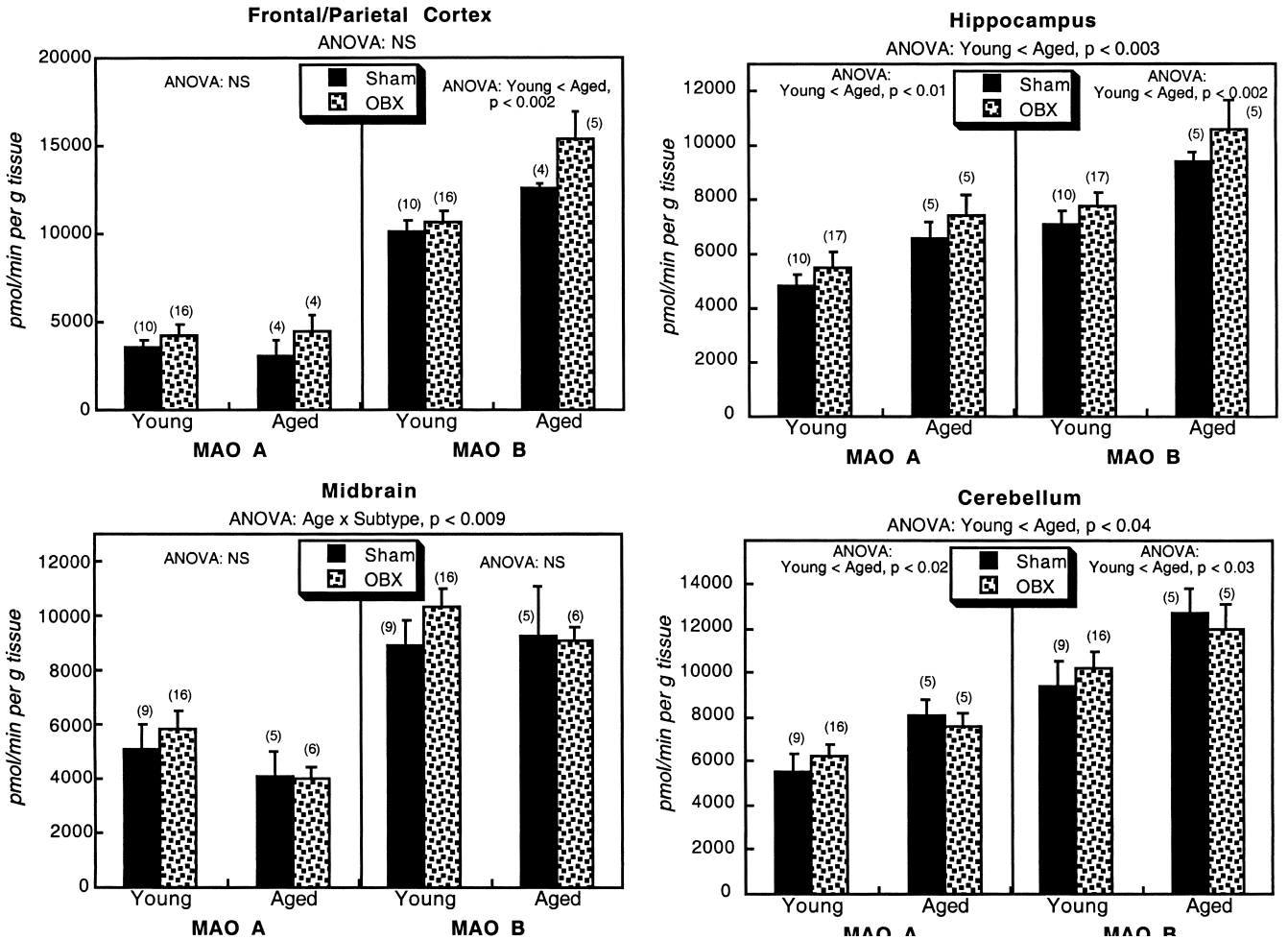

Fig. 1. Activities of MAO subtypes in young and aged animals subjected to sham surgery or olfactory bulbectomy (OBX). Data represent means and standard errors of the number of animals shown in parentheses. ANOVA across both age groups, both treatments and both subtypes appears at the top of each panel and ANOVA for each subtype appears within the panels. Post-hoc tests of individual differences between sham and OBX groups was not carried out because of the absence of age×OBX interactions; accordingly, only main effects are indicated.

evidenced by failure to suppress adrenocortical activity upon acute challenge with dexamethasone [3,4,18,21, 23,27,34]. Elderly depressives with HPA dysregulation are specifically the ones who maintain better responses to monoamine reuptake inhibitors, both biochemically [28] and in terms of clinical response [11]. In the rat OBX model of depression, HPA axis function is maintained [35]; accordingly, our studies specifically model the subpopulation of elderly depressives who maintain HPA axis function and who are less responsive to tricyclic antidepressants or serotonin reuptake inhibitors [11]. The present results, which show that the OBX lesion does not further enhance MAO activity, implies that the therapeutic response to MAO inhibitors is likely to be maintained in geriatric depressives who have poor responses to monoamine reuptake inhibitors. In depressed patients, platelet MAO is likewise responsive to abnormal HPA axis status [9,20], so that the OBX model may prove useful in validating biological markers that can distinguish subgroups that do or do not respond to specific drug therapies [11,28]. Notwithstanding the importance of identifying patients who are likely to show better antidepressant responses with MAO inhibitors as compared to monoamine reuptake inhibitors, there are advantages and limitations inherent in the use of MAO inhibitors that are specific to the geriatric population. Unlike reuptake inhibitors, these agents are not anticholinergic and thus do not exacerbate the cognitive impairment that accompanies senescence [19,25]. On the other hand, MAO inhibitors are likely to be unsuitable where there is underlying cardiovascular disease, such as hypertension.

Although OBX lesioning failed to alter MAO subtypes in young or aged rats, there were clear-cut differences between the two groups in the structural consequences of the lesion. The aged OBX group showed greater atrophy of the frontal/parietal cortex than did young OBX rats, suggesting augmented neurodegeneration. The age-dependent deficit was also detected in the midbrain, a region far more distal to the lesion. The olfactory bulbs have major projections to sites in the forebrain, and through inter-

connections in the amygdala, to the midbrain [10], so that the targeting of these two regions likely represents pathway-specific atrophy rather than simply a nonspecific neurodegeneration in aged animals. It is notable that the midbrain is the same region that showed no age-dependent changes in MAO activity; while it is tempting to speculate that naturally-occurring neurodegeneration may offset the specific overexpression of MAO in this region, proof of that connection requires further study. On the other hand, there was no corresponding atrophy of the hippocampus in aged OBX rats, despite the fact that this region is particularly susceptible to stress-induced degeneration in the aging brain [13,26]; also, the hippocampus showed a significant increase in MAO activity with age, unlike the midbrain. Future work should address the functional consequences of targeted neurodegeneration in the aging brain to determine if the behavioral differences noted between young and aged OBX rats reflect the selective loss of frontal/parietal and midbrain projections in the aged animals, and to determine if neurodegeneration has a significant impact on monoamine function via changes in MAO activity.

Acknowledgements

The authors thank Clay Ramsey for technical assistance. Research supported by USPHS HD09713.

References

[1] G.K. Aghajanian, D.S. Charney, R.S. Duman, G.R. Heninger, Neurobiology of Affective Disorders, Raven Press, New York, 1993.
[2] Y. Arai, H. Kinemuchi, Differences between monoamine oxidase concentrations in striatum and forebrain of aged and young rats, J. Neural Transm. 72 (1988) 99–105.
[3] R.J. Branconnier, G.F. Oxenkrug, I. McIntyre, N. Pomara, N.E. Harto, S. Gershon, Prediction of serum cortisol response to dexamethasone in normal volunteers: a multivariate approach, Psychopharmacology 84 (1984) 274–275.
[4] B.J. Carroll, G.C. Curtis, J. Mendels, Cerebrospinal fluid and plasma free cortisol concentrations in depression, Psychol. Med. 6 (1976) 235–244.
[5] B.J. Carroll, M. Feinberg, J.F. Greden, J. Tarika, A.A. Albala, R.F. Haskett et al., A specific laboratory test for the diagnosis of melancholia, Arch. Gen. Psychiat. 38 (1981) 15–22.
[6] G. Cvijic, R. Radojicic, J. Djordjevic, V. Davidovic, The effect of glucocorticoids on the activity of monoamine oxidase, copper–zinc superoxide dismutase and catalase in the rat hypothalamus, Funct. Neurol. 10 (1995) 175–181.
[7] Danish University Antidepressant Group, Citalopram: clinical effect profile in comparison with clomipramine: a controlled multicenter study, Psycopharmacology (Berlin) 90 (1986) 131–138.
[8] Danish University Antidepressant Group, Paroxetine: a selective serotonin reuptake inhibitor showing better tolerance, but weaker antidepressant effect than clomipramine in a controlled multicenter study, J. Affect. Disord 18 (1990) 289–299.
[9] A. Georgotas, R.E. McCue, E. Friedman, W.E. Hapworth, O.M. Kim, T.B. Cooper et al., Relationship of platelet MAO activity to characteristics of major depressive illness, Psychiat. Res. 19 (1986) 247–256.
[10] J.P. Kelly, A.S. Wrynn, B.E. Leonard, The olfactory bulbectomized rat as a model of depression: an update, Pharmacol. Therap. 74 (1997) 299–316.
[11] N.M.K.N.Y. Kin, N.P.V. Nair, M. Amin, G. Schwartz, S.K. Ahmed, P. Holm et al., The dexamethasone suppression test and treatment outcome in elderly depressed patients participating in a placebo-controlled multicenter trial involving moclobemide and nortriptyline, Biol. Psychiat. 42 (1997) 925–931.
[12] B.E. Leonard, M. Tuite, Anatomical, physiological and behavioral aspects of olfactory bulbectomy in the rat, Intl. Rev. Neurobiol. 22 (1981) 251–286.
[13] B.S. McEwen, Re-examination of the glucocorticoid hypothesis of stress and aging, Prog. Brain Res. 93 (1992) 365–380.
[14] B. Meister, H. Johnson, B. Ulfhake, Increased expression of serotonin transporter messenger RNA in raphe neurons of the aged rat, Mol. Brain Res. 33 (1995) 87–96.
[15] D.L. Murphy, C. Wright, M. Buchsbaum, A. Nichols, J.L. Costa, R.J. Wyatt, Platelet and plasma amine oxidase activity in 680 normals: sex and age differences and stability over time, Biochem. Med. 16 (1976) 254–265.
[16] N.P. Nair, S.K. Ahmed, N.M. Kin, T.E. West, Reversible and selective inhibitors of monoamine oxidase A in the treatment of depressed elderly patients, Acta Psychiat. Scand. 386 (1995) 28–35.
[17] J.C. Nelson, C.M. Mazure, P.I. Jatlow, Desipramine treatment of major depression in patients over 75 years of age, J. Clin. Psychopharmacol. 15 (1995) 99–105.
[18] G.F. Oxenkrug, I.M. McIntyre, M. Stanley, S. Gershon, Dexamethasone suppression test: experimental model in rats and effect of age, Biol. Psychiat. 19 (1984) 413–416.
[19] T.E. Oxman, Antidepressants and cognitive impairment in the elderly, J. Clin. Psychiat. 57 (Suppl 5) (1996) 38–44.
[20] G.N. Pandey, R.P. Sharma, P.G. Janicak, J.M. Davis, Monoamine oxidase and cortisol response in depression and schizophrenia, Psychiat. Res. 44 (1992) 1–8.
[21] B. Pfohl, B. Sherman, J. Schlechte, R. Stone, Pituitary–adrenal axis rhythm disturbances in psychiatric depression, Arch. Gen. Psychiat. 42 (1985) 897–903.
[22] K.J. Reinikainen, L. Paljarvi, T. Halonen, O. Malminen, V.M. Kosma, M. Laakso et al., Dopaminergic system and monoamine oxidase-B activity in Alzheimer's disease, Neurobiol. Aging 9 (1988) 245–252.
[23] J.C. Ritchie, R.L. Scotch, C.B. Nemeroff, B.J. Carroll, The effect of age on DST status and plasma dexamethasone concentration in depressed patients, Biol. Psychiat. 27 (1990) 45A–46A.
[24] S.P. Roose, A.H. Glassman, E. Attia, S. Woodring, Comparative efficacy of selective serotonin reuptake inhibitors and tricyclics in the treatment of melancholia, Am. J. Psychiat. 151 (1994) 1735–1739.
[25] C. Salzman, Clinical guidelines for the use of antidepressant drugs in geriatric patients, J. Clin. Psychiat. 46 (1985) 38–45.
[26] R.M. Sapolsky, The physiological relevance of glucocorticoid endangerment of the hippocampus, Ann. NY Acad. Sci. 746 (1994) 294–304.
[27] R.P. Sharma, G.N. Pandey, P.G. Janicak, J. Peterson, J.E. Comaty, J.M. Davis, The effect of diagnosis and age on the DST: a meta-analytic approach, Biol. Psychiat. 24 (1988) 555–568.
[28] T.A. Slotkin, J.C. Hays, C.B. Nemeroff, B.J. Carroll, Dexamethasone Suppression Test identifies a subset of elderly depressed patients with reduced platelet serotonin transport and resistance to imipramine inhibition of transport, Depression and Anxiety 6 (1997) 19–25.
[29] T.A. Slotkin, D.B. Miller, F. Fumagalli, E.C. McCook, J. Zhang, G. Bissette et al., Modeling geriatric depression in animals: biochemical and behavioral effects of olfactory bulbectomy in young versus aged rats, J. Pharmacol. Exp. Ther. 289 (1999) 334–345.

[30] T.A. Slotkin, F.J. Seidler, J.C. Ritchie, Effects of aging and glucocorticoid treatment on monoamine oxidase subtypes in rat cerebral cortex: therapeutic implications, Brain Res. Bull. 47 (1998) 345–348.
[31] T.A. Slotkin, W.L. Whitmore, G.A. Barnes, K.R.R. Krishnan, D.G. Blazer, D.L. Knight et al., Reduced inhibitory effect of imipramine on radiolabeled serotonin uptake into platelets in geriatric depression, Biol. Psychiat. 25 (1989) 687–691.
[32] G.W. Snedecor, W.G. Cochran, Statistical Methods, Iowa State University Press, Ames, Iowa, 1967.
[33] D.L. Sparks, V.M. Woeltz, W.R. Markesbery, Alterations in brain monoamine oxidase activity in aging Alzheimer's disease, and Pick's disease, Arch. Neurol. 48 (1991) 718–721.
[34] D. Stangl, B. Pfohl, M. Zimmerman, W. Coryell, C. Corenthal, The relationship between age and post-dexamethasone cortisol: a test of three hypotheses, J. Affective Dis. 11 (1986) 185–197.
[35] H. van Riezen, B.E. Leonard, Effects of psychotropic drugs on the behavior and neurochemistry of olfactory bulbectomized rat, Pharmacol. Ther. 47 (1990) 21–34.
[36] J.W. Veals, C.A. Korduba, S. Symchowicz, Effect of dexamethasone on monoamine oxidase inhibition by iproniazid in rat brain, Eur. J. Pharmacol. 41 (1977) 291–299.
[37] J.L. Venero, C. de la Roza, A. Machado, J. Cano, Age-related changes on monoamine turnover in hippocampus of rats, Brain Res. 631 (1993) 89–96.