

Serotonergic Cell Signaling in an Animal Model of Aging and Depression: Olfactory Bulbectomy Elicits Different Adaptations in Brain Regions of Young Adult vs Aging Rats

Theodore A Slotkin^{*1}, Mandy M Cousins¹, Charlotte A Tate¹ and Frederic J Seidler¹

¹Department of Pharmacology & Cancer Biology, Duke University Medical Center, Durham, NC, USA

Aging involves neuronal and synaptic loss, and maintenance of function depends on adaptations in cellular responsiveness. We studied olfactory bulbectomy (OBX), a model that recapitulates monoaminergic dysfunction in depression, in 10-week vs 19-month-old rats, and evaluated 5HT (5-hydroxytryptamine, serotonin) mechanisms. OBX elicited little change in 5HT_{1A} receptors in the cerebral cortex or striatum of either age group. In contrast, 5HT₂ receptors showed disparate effects, with a decrease in the cerebral cortex of young OBX but not aging OBX rats, whereas the latter group showed a selective decrease in striatal 5HT₂ receptors. Greater differences were apparent for 5HT-mediated cell signaling, assessed for the adenylyl cyclase (AC) cascade. In young animals, 5HT had a stimulatory effect on AC that was unaltered by OBX. However, in aging animals, the pattern of 5HT responses showed marked alterations in response to OBX: under basal conditions, stimulatory effects were enhanced but when AC was activated with forskolin, 5HT became markedly inhibitory in the striatum of aged OBX animals. Assessment of the relative AC responses to two direct stimulants that act on different epitopes of the enzyme, forskolin and Mn²⁺, pointed to a shift in the AC isoform and/or its ability to associate with G-proteins as the mechanism underlying the age-related differences for OBX effects. These data indicate that there are biological distinctions in the response of 5HT systems to OBX in young adult vs aging animals, which, if present in geriatric depression, could provide a mechanistic basis for differences in responses to antidepressants that act on 5HT.

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INTRODUCTION

Neuronal loss and consequent deficits in synaptic function are a hallmark of aging and its associated neuropathologies, such as neurodegenerative diseases and stroke. The ability of the brain to adapt to neuronal or synaptic loss requires plasticity at the level of cell signaling and it is increasingly evident that monoaminergic neurotransmitters play a key role in this process. Stimulation of monoaminergic receptors can offset aging-related deterioration of behavioral performance (Coull, 1994) and recovery after surgical lesions depends on adaptive changes in monoamine systems (Goldstein and Bullman, 1997). Importantly, this situation changes drastically in the aging brain: lesions that compromise catecholamine (Sirvio *et al*, 1991; Slotkin *et al*, 2000a) or 5HT (5-hydroxytryptamine,

serotonin) (Slotkin *et al*, 2000a) pathways produce a different spectrum of synaptic and behavioral effects from those seen after lesioning of the young brain. It is now evident that impaired adaptability of the aging brain to the loss of monoaminergic inputs is critical to geriatric depression. Elderly depressives are less responsive to monoamine reuptake inhibitors, the mainstay of antidepressant therapy (Danish University Antidepressant Group, 1990; Nelson *et al*, 1995; Roose *et al*, 1994). Although some of these differences undoubtedly reflect pharmacokinetic factors and increased susceptibility to side effects in the geriatric population, recent studies suggest that there is a biological difference in the response of the aging brain to serotonin-specific reuptake inhibitors (SSRIs). Senescent rats are resistant to SSRI-induced behavioral effects (Stanford *et al*, 2002) and these agents are also less effective in aged monkeys (Kakiuchi *et al*, 2001). Using 5HT uptake into blood platelets as a surrogate marker for CNS actions, we found that the 5HT transporter in a subset of elderly depressed patients (those showing a normal cortisol suppression response upon challenge with dexamethasone) was subresponsive to imipramine (Slotkin *et al*, 1989, 1997).

*Correspondence: Dr TA Slotkin, Box 3813/Department of Pharmacology & Cancer Biology, Duke University Medical Center, Durham, NC 27710 USA, Tel: +1 919 681 8015, Fax: +1 919 684 8197, E-mail: t.slotkin@duke.edu

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Olfactory bulbectomy (OBX) provides one of the most useful models for the study of CNS lesions that contribute to depression-like outcomes. In the rat, OBX produces behavioral and biochemical characteristics that, as in human depression, are reversed after chronic, but not acute, antidepressant therapy (for a review see Kelly *et al*, 1997; Leonard and Tuite, 1981), and importantly, these animals exhibit abnormalities of monoaminergic function parallel to those thought to operate in depression (van Riezen and Leonard, 1990). In previous work, we established that, in aging rats, OBX lesions produce changes in 5HT transporter expression opposite to those seen in young rats given the same lesion (Slotkin *et al*, 1999b). Equally important, whereas young OBX rats showed adaptive changes in cell signaling responses to norepinephrine and dopamine that would compensate for decreased presynaptic function, aging OBX rats showed either no adaptation or even impairment of signaling, a distinctly maladaptive response (Slotkin *et al*, 1999b). Subsequent studies with chemical lesions showed that the lack of compensatory increases in signaling responses is a general characteristic of the aging brain, rather than representing an impairment specific to the OBX model (Dugar *et al*, 2001; Slotkin *et al*, 2000a). To date, however, there has been no exploration of the impact of the OBX lesion in the aging brain on 5HT receptors or their ability to evoke cellular responses, and the current study addresses that issue by comparing the effects of OBX in rats lesioned at 10 weeks vs 19 months of age. We selected 19 months rather than examining the extreme of the lifespan; neurodegeneration, synaptic dysmorphology, and neuronal loss are likely to be present when very old rats are used, obscuring or exacerbating any primary effects of the lesion on cellular function (Meister *et al*, 1995).

Assessments focused first on two 5HT receptor populations, 5HT_{1A} and 5HT₂, in two distinct regions containing 5HT projections (cerebral cortex, striatum). Regionally selective shifts in the expression of these two subtypes are characteristic in human depression (Anand and Charney, 2000; Arango *et al*, 2001; Fujita *et al*, 2000; Leonard, 2000; Nemeroff, 1998; Yatham *et al*, 1999, 2000) and animal models of depression (Leonard, 2000; Osterlund *et al*, 1999; Overstreet *et al*, 1992). For assessment of cell signaling responses, we focused on adenylyl cyclase (AC), the enzyme responsible for generation of cyclic AMP. The two 5HT receptor subtypes evaluated here converge on the control of AC through both stimulatory and inhibitory mechanisms. 5HT_{1A} receptors inhibit AC through the inhibitory G-protein, G_i (Barnes and Sharp, 1999), and also can stimulate AC through release of G-protein $\beta\gamma$ subunits (Raymond *et al*, 1999). 5HT₂ receptor stimulation leads to heterologous enhancement of AC responses mediated by other (ie non-5HT) receptors (Morin *et al*, 1992; Rovescalli *et al*, 1993), and 5HT₇ receptors, which crossreact with ligands for 5HT_{1A} receptors, are stimulatory for AC (Duncan *et al*, 1999; Lovenberg *et al*, 1993). Accordingly, we evaluated the net balance of the AC response to 5HT so as to integrate all these disparate signals. In addition, we determined the potential interaction of OBX and aging on components of AC signaling downstream from the receptors; our earlier work indicated that the age-related differences in signaling adaptations to lesions are 'heterologous', that is, shared by multiple receptor inputs, reflecting alterations at a post-

receptor locus, including the induction of AC itself (Slotkin *et al*, 1999b, 2000a). Therefore, we assessed the response to two AC stimulants, forskolin and Mn²⁺, that bypass the need for receptor activation. As the two stimulants act at different epitopes on the AC molecule, the preference for one over the other reflects shifts in molecular conformation, primarily influenced by the AC isoform and/or expression and function of G-protein components that link receptor activation to AC activity (Zeiders *et al*, 1999b). For the cell signaling studies, we extended our observations to include a brain region comprising 5HT cell bodies (brainstem) in order to determine if alterations in 5HT responses are generalized throughout the CNS, similar to our approach in earlier work with this model (Slotkin *et al*, 1999b). In human depression, regulatory changes in 5HT systems differ substantially between terminal zones and areas containing the cell bodies (Fujita *et al*, 2000; Stockmeier *et al*, 1998).

METHODS

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) 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. At 1 week after arrival, animals were anesthetized with 6.5 mg/kg of xylazine and 44 mg/kg of ketamine, given intraperitoneally (i.p.). 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 (Kelly *et al*, 1997; Leonard and Tuite, 1981). 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. Each of the four groups (young sham operated, young OBX, old sham operated, old OBX) contained six animals.

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 occipital and temporal lobes of the cerebral cortex, the striatum, and the brainstem (Slotkin *et al*, 1999b, 2000a). Brain regions were frozen in liquid nitrogen and maintained at –45°C until used.

5HT Receptor Binding

Tissues were thawed and homogenized (Polytron, Brinkmann Instruments, Westbury, NY) in ice-cold 50 mM Tris

(pH 7.4), and the homogenates were sedimented at 40 000g for 15 min. The pellets were washed twice by resuspension (Polytron) in homogenization buffer followed by resedimentation, and were then dispersed with a homogenizer (smooth glass fitted with Teflon pestle) in the same buffer.

Two radioligands (Perkin-Elmer Life Sciences, Boston, MA) were used to determine 5HT receptor binding (Xu *et al*, 2002): 1 nM [³H]8-hydroxy-2-(di-*n*-propylamino)tetralin (specific activity, 135 Ci/mmol) for 5HT_{1A} receptors (Park *et al*, 1999; Stockmeier *et al*, 1998) and 0.4 nM [³H]ketanserin (specific activity, 63 Ci/mmol) for 5HT₂ receptors (Leysen *et al*, 1982; Park *et al*, 1999). For 5HT_{1A} receptors, incubations lasted for 30 min at 25°C in a buffer consisting of 50 mM Tris (pH 8), 0.5 mM MgCl₂, and 0.5 mM sodium ascorbate; 100 μM 5HT (Sigma Chemical Co, St Louis, MO) was used to displace specific binding. For 5HT₂ receptors, incubations lasted 15 min at 37°C in 50 mM Tris (pH 7.4) and specific binding was displaced with 10 μM methylsergide (Sandoz Pharmaceuticals, E. Hanover, NJ). Incubations were stopped by the addition of excess of ice-cold buffer and the labeled membranes were trapped by rapid vacuum filtration onto glass fiber filters that were presoaked in 0.15% polyethyleneimine (Sigma). The filters were then washed repeatedly and radiolabel was determined.

AC Activity

The same membrane preparation was used as for receptor binding assays; the methods have been described in detail previously (Slotkin *et al*, 1990, 1992, 2001; Xu *et al*, 2002). Membrane aliquots were incubated for 10 min at 30°C with final concentrations of 40 mM Tris-HCl (pH 7.4), 10 mM theophylline, 1 mM adenosine 5'-triphosphate, 10 μM guanosine 5'-triphosphate, 2 mM MgCl₂, 1 mg/ml bovine serum albumin, and a creatine phosphokinase-ATP-regenerating system consisting of 10 mM sodium phosphocreatine and 8 IU/ml phosphocreatine kinase, in a total volume of 250 μl (all reagents from Sigma). The enzymatic reaction was stopped by placing the samples in a 90–100°C water bath for 5 min, followed by sedimentation at 3000g for 15 min, and the supernatant solution was assayed for cyclic AMP by radioimmunoassay (Amersham Biosciences, Piscataway, NJ). The AC response to 5HT was evaluated in two different ways: the effect of 100 μM 5HT on basal activity, and the effect of 5HT on the response to 100 μM forskolin (Sigma), which acts directly on AC, bypassing the need for the activation of neurotransmitter receptors (Seamon and Daly 1986). This approach enables detection of potential inhibitory or excitatory actions (Chow *et al*, 2000; Slotkin *et al*, 1999a; Xu *et al*, 2002). Comparisons between AC responses to forskolin and Mn²⁺ were carried out at concentrations of 100 μM and 10 mM, respectively. The concentrations of all the agents used here have been found previously to be optimal for effects on AC and/or were confirmed in preliminary experiments (Auman *et al*, 2000, 2001; Xu *et al*, 2002; Zeiders *et al*, 1997, 1999a).

Data Analysis

Results are presented as means and standard errors. Lesion-related differences were analyzed first by a global analysis

of variance (ANOVA) incorporating all variables: age, surgical treatment, and brain region; because values in the different regions or ages differed by substantial amounts, data were log-transformed to correct for heterogeneous variance. Where this initial test indicated an interaction between lesioning and age, lower-order ANOVAs were carried out to identify significant main effects in each age group. Where lesioning interacted with both age and region, Fisher's protected least significant difference was used to identify differences in the individual regions, which then did not require log transformation. Significance for all tests was assumed at $p < 0.05$.

RESULTS

The general effects of aging and OBX lesions on body and brain region weights were reported earlier (Slotkin *et al*, 1999b, 2000b). Although OBX lesions evoked initial body weight deficits in both age groups, by 25 days later, neither were the weights distinguishable between sham-operated and lesioned animals nor were there any lesion-related significant differences in weights of the three brain regions (data not shown). OBX had little or no effect on 5HT_{1A} receptor binding in either age group but elicited regionally disparate effects on 5HT₂ receptors in young vs aging rats (Figure 1). In young animals, there was a small (12%) but significant reduction in cerebrocortical 5HT₂ receptors, whereas the older animals showed a smaller change that did not achieve significance. Age-related differences in the effects of OBX were more prominent in the striatum. Young animals showed no alteration in response to the lesion, whereas older animals showed a marked (35%) reduction.

Across all regions and groups, the net effect of 5HT on basal AC activity was stimulatory ($p < 0.0001$) (Figure 2a). In the young group, the stimulatory pattern was significant overall ($p < 0.006$) and did not show any net effect of lesioning. In contrast, older controls did not show significant stimulatory responses to 5HT and the OBX lesion produced a robust and significant increase in the response. When the 5HT response was superimposed on forskolin-stimulated AC activity, a different age-selective pattern emerged (Figure 2b). In the young group there was again a net stimulation evoked by 5HT ($p < 0.05$) without any alteration evoked by OBX lesioning. In the older animals, however, the lesion elicited a profound switch to a net inhibitory effect in the striatum.

As the AC response to receptor stimulants is influenced by G-protein coupling and AC isoforms downstream from neurotransmitter receptors, we also compared the effects of OBX lesions on the proportional response of AC to forskolin and Mn²⁺ (Figure 3). There were large, inherent regional differences in the response ratio, with Mn²⁺ producing greater stimulation in the cerebral cortex and brainstem, whereas forskolin elicited a much larger response in the striatum. The OBX lesion had little or no effect on AC response preference for forskolin or Mn²⁺ in young animals but elicited a significant shift toward Mn²⁺ in the older OBX group, with the greatest magnitude of effect in the striatum.

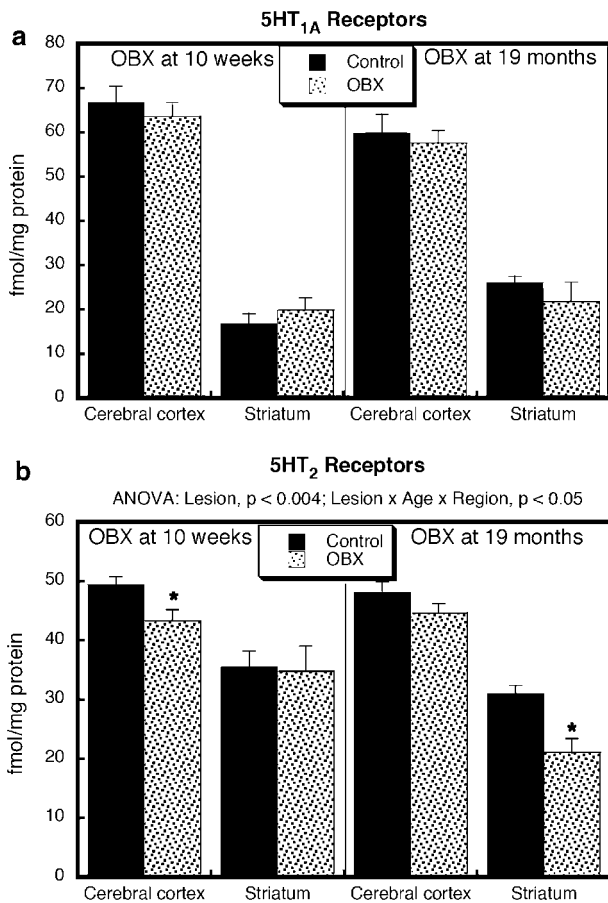


Figure 1 Effects of OBX on 5HT_{1A} (a) and 5HT₂ (b) receptor binding. Data represent means and standard errors obtained from six animals in each group. ANOVA across regions and age groups appears at the top of each panel. Asterisks denote individual values that differ from the age-matched control group, evaluated only where the ANOVA indicated a significant interaction of lesioning with the other two variables.

DISCUSSION

Results of the current study indicate that aging dramatically alters the adaptive synaptic changes in response to lesions that impair 5HT presynaptic activity. Importantly, these disparities are not apparent with aging alone, as there was little or no difference between young and aging rats in the patterns of 5HT receptor expression or 5HT-mediated responses without the OBX lesion. Our findings thus are consonant with the idea that functional impairment associated with aging can be revealed by challenges that require adaptive responses.

In our earlier work with the OBX model and with chemical lesions of monoamine systems, we found heterologous changes in AC signaling that differed with age (Slotkin *et al*, 1999b, 2000a): in young animals, the lesions elicited global induction of AC itself, and a consequent increase in stimulatory responses to norepinephrine and dopamine; in aging animals, these changes were either absent or in some brain regions, opposite to those seen in young animals, and were superimposed on the general increase in AC activity associated with aging by itself (Palego *et al*, 2000; Slotkin *et al*, 1999b). Similarly, the current results for 5HT cell signaling are consonant with

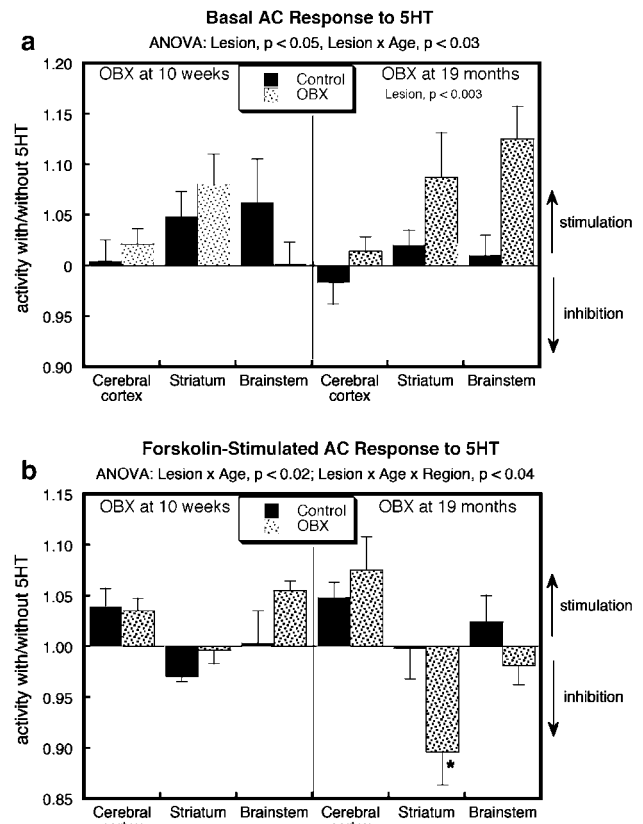


Figure 2 Effects of OBX on the AC response to 5HT. The response was assessed as the ratio of activity in the presence of 5HT to activity without 5HT, determined either under basal conditions (a) or in the presence of the direct AC stimulant, forskolin (b). ANOVA across regions and age groups appears at the top of each panel. In (a), lower-order ANOVAs (shown within the panel) were carried out for young and aging rats because of the interaction of lesion \times age; tests of effects in individual regions were not conducted because of the absence of a treatment \times region interaction. In (b), where there was an interaction of lesion \times age \times region, the asterisk denotes the individual value that differs from the age-matched control group.

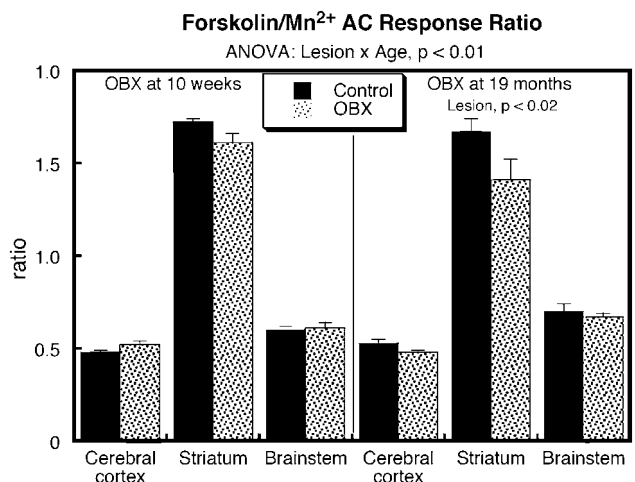


Figure 3 Effects of OBX on the relative AC response to forskolin and Mn²⁺ in brain regions of young and aging rats. ANOVA across regions and age groups appears at the top of the panel. Lower-order ANOVAs for each age group (shown within the panel) were carried out because of the interaction of lesion \times age; tests of effects in individual regions were not conducted because of the absence of a treatment \times region interaction.

heterologous changes downstream from 5HT receptors. The most notable age-related difference in the effects of OBX lesioning on 5HT receptors was the disparity in the suppression of the 5HT₂ subtype in the striatum, which occurred in the aging OBX group but not in the young OBX animals. Although 5HT₂ receptors are not linked directly to AC activity, they act indirectly to enhance the coupling of the stimulatory G-protein, G_s, to stimulatory receptors, producing heterologous AC stimulation (Morin et al, 1992; Rovescalli et al, 1993). Accordingly, the decrement in 5HT₂ receptors in the older OBX group should have elicited a global shift in responses away from stimulation and toward inhibition. Instead, the response to 5HT under basal conditions showed enhancement, implying that the major changes are at the level of signaling elements downstream from the receptors themselves.

In keeping with this interpretation, when the response to 5HT was evaluated in the presence of forskolin, a direct AC stimulant, the aging animals showed a specific defect that was not present in the young OBX group, a profound shift in the 5HT response in the striatum toward inhibition. The differing results for the effects of 5HT receptor activation on basal AC and on forskolin-stimulated AC thus suggested to us that the unique effects of the OBX lesion in the older animals reflected alterations in AC itself, either in the isoform being expressed or in the association between AC and G-proteins (Chaudhry et al, 1996; Gao et al, 1998, 1999; Scarpace et al, 1996; Zeiders et al, 1999b), which is influenced by factors such as phosphorylation (Premont et al, 1992). This explanation was then verified by assessing the ratio of AC responses to forskolin vs Mn²⁺, two stimulants that act at different AC epitopes and whose relative activities therefore reflect the AC isoform and the effectiveness of G-protein association with AC (Zeiders et al, 1999b). Across both age groups, with or without the OBX lesion, the striatum differed radically from the cerebral cortex or brainstem, displaying a much higher preference for forskolin. With introduction of the lesion, only the aging group showed a significant drop in the forskolin preference, indicative of the predicted postreceptor alteration in AC signaling. These findings are thus in keeping with earlier results pointing to heterologous changes downstream from the receptors as the site of dysregulation in the response to lesioning in the aging brain (Slotkin et al, 1999b, 2000a).

In conclusion, our results indicate that the adaptations of 5HT systems to OBX lesions differ radically between the young and aging brain, disparities that are not readily apparent under basal conditions, but that emerge upon neuronal damage. Equally important, the discrepant responses reflect cellular events that are downstream from 5HT receptors and are thus likely to be shared by numerous neurotransmitter inputs. The divergence seen here for 5HT systems is thus likely to represent only a small part of a larger spectrum of age-related differences in the response to changes in synaptic activity elicited by lesions, drugs, or environmental challenges. Accordingly, our results may provide a biological explanation for some of the differences seen in the response to SSRIs or other antidepressant drugs in geriatric depression as compared to depression earlier in life.

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