Modeling Geriatric Depression in Animals: Biochemical and Behavioral Effects of Olfactory Bulbectomy in Young Versus Aged Rats

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ABSTRACT

Geriatric depression exhibits biological and therapeutic differences relative to early-onset depression. We studied olfactory bulbectomy (OBX), a paradigm that shares major features of human depression, in young versus aged rats to determine mechanisms underlying these differences. Young OBX rats showed locomotor hyperactivity and a loss of passive avoidance and tactile startle. In contrast, aged OBX animals maintained avoidance and startle responses but showed greater locomotor stimulation; the aged group also exhibited decreased grooming and suppressed feeding with novel presentation of chocolate milk, effects which were not seen in young OBX. These behavioral contrasts were accompanied by greater atrophy of the frontal/parietal cortex and midbrain in aged OBX. Serotonin transporter sites were increased in the cortex and hippocampus of young OBX rats, but were decreased in the aged OBX group. Cell signaling cascades also showed age-dependent effects, with increased adenylyl cyclase responses to monoaminergic stimulation in young OBX but no change or a decrease in aged OBX. These data indicate that there are biological distinctions in effects of OBX in young and aged animals, which, if present in geriatric depression, provide a mechanistic basis for differences in biological markers and drug responses. OBX may provide a useful animal model with which to test therapeutic interventions for geriatric depression.

Geriatric depression presents an unique set of physiological and biochemical problems that have an adverse impact on therapeutics and outcome. In particular, this patient population displays a poor response to serotonin-specific reuptake inhibitors, the mainstay of depression therapy (Carroll et al., 1981; Aghajanian et al., 1993). Although many reports claim that serotonin-specific antidepressants are equivalent to the tricyclic antidepressants, only phenelzine, nortriptyline, and imipramine have a proven efficacy as a tricyclic antidepressant action (Schneider, 1993; Volz and Moller, 1994), and in fact, the serotonin-specific reuptake inhibitors have not been evaluated adequately in the elderly population, where a relatively high rate of response to placebo is known to occur (Wilcox et al., 1992). These agents, as well as desipramine, have failed to show satisfactory efficacy in geriatric depression (Danish University Antidepressant Group, 1986, 1990; Roose et al., 1994; Nelson et al., 1995).

In part, the differences between responses in elderly and young depressed patients may reflect regulatory differences in the hypothalamus-pituitary-adrenal (HPA) axis. The elderly depressed population shows twice the rate of dexamethasone nonsuppression in association with a greater general severity of the disease (Ritchie et al., 1990). There is close, mutual regulation of serotonergic systems and glucocorticoids (Arora and Meltzer, 1986; Jøels and De Kloet, 1991; Pepin et al., 1992), a relationship pursued in a number of investigations of geriatric depression. Using inhibition of platelet serotonin uptake as an index of tricyclic antidepressant action, we recently found that elderly nonsuppressors maintained their imipramine effect, whereas suppressors did not (Slotkin et al., 1997a); simultaneously, clinical evaluations demonstrated that nonsuppression could be used as a predictor of antidepressant therapeutic outcome in geriatric depression (Kin et al., 1997). Indeed, in controlled clinical trials with the dexamethasone suppression test, suppressors have a very low rate of specific response to tricyclic antidepressants (approximately 10% above placebo response rates), whereas nonsuppressors have a specific response rate of approximately 60% because of their low rate of placebo re-
response (Carroll, 1989). These results indicate that elderly depression may be a biologically distinct disorder with different underlying neurochemical alterations and, hence, a different therapeutic prospectus. Comparing young and aged rats, we have demonstrated that elevated glucocorticoid levels induce different sets of responses for serotonin transporter expression and function (Fumagalli et al., 1996; Slotkin et al., 1997b), and at the same time, produce changes in postsynaptic cell-signaling cascades that influence not only serotonergic, but also catecholaminergic neurotransmission (Slotkin et al., 1996). These results suggest that HPA axis abnormalities and their impact on serotonergic system are just one component of the age-related effects contributing to biological differences between young and elderly depressives.

If, as shown, poor therapeutic outcome is a characteristic of depressed geriatric patients who maintain normal HPA axis reactivity (Kin et al., 1997; Slotkin et al., 1997a), then an animal model of elderly depression that maintains HPA axis integrity would provide a means of distinguishing between the underlying biological differences related to aging of the brain as opposed to those that are glucocorticoid-related. The olfactory bulbectomized (OBX) rat exhibits behavioral and biochemical characteristics that, as in human, are reversed after chronic, but not acute, antidepressant therapy (reviews, Leonard and Tuite, 1981; Kelly et al., 1997). Importantly for our studies, these animals maintain dexamethasone suppression while developing abnormalities of serotonergic and catecholaminergic function that, along with the behavioral abnormalities, resolve with drugs affecting either of these transmitter systems (van Riezen and Leonard, 1990).

Although previous studies have compared OBX effects in juvenile and adult rats (Broekkamp et al., 1986), to our knowledge, no one has explored the OBX model in aging. We have undertaken a comprehensive behavioral and biochemical comparison of the effects of OBX in young and aged rats, with neurochemical determinations focusing on factors that we have found previously to respond differently with age (Slotkin et al., 1989, 1996, 1997a,b; Fumagalli et al., 1996): serotonin transporter expression in brain regions and platelets, and adenylyl cyclase signaling mechanisms and their response to catecholamines. Neurodegeneration, synaptic dysmorphism, and neuronal loss are likely to be present.
and aspirated according to established protocols (Leonard and Tuite, 1981; Kelly et al., 1997). The cavity was packed with surgical foam, the skin was closed with surgical clips and bupivacaine was applied. The animals were given 40,000 IU/kg of procaine penicillin i.m., 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. After surgery, animals were handled and weighed daily.

Experiments were carried out 3 weeks after surgery. Behavior was tested over a 3-day span after which the same animals were used for biochemical determinations. Behavioral tests were recorded on videotape and scored by a blinded observer. Between 9:00 and 11:00 AM on the first day of testing, we evaluated tactile startle (Knapp and Pohorecky, 1995). The home cage was moved to a testing area and after a 1-min habituation period, an air puff was applied to the back of the neck; scoring categorized animals according to freeze times of greater or less than 3 s. At 7:00 PM the same day (1 h after the start of the dark cycle), animals were presented with chocolate milk to assess novelty-suppressed feeding (Mufson et al., 1976; Pucilowski et al., 1993). All food was removed and bottles containing water or chocolate milk were presented to the animals for a 2-h period.

On the second day of testing, open field activity was determined between 9:00 AM and 12:00 PM. Each animal was videotaped for a 5-min period in a circular field (90-cm diameter × 45-cm height) divided into 10-cm squares; the floor and walls of the apparatus and room were black and the test area was illuminated brightly. Scoring included horizontal and vertical activity, fecal boli, and grooming. In the afternoon (1:00 to 3:00 PM), animals were trained for step-through passive avoidance (van Riezen and Leonard, 1990; Kelly et al., 1997) using the Gemini Avoidance System (San Diego Instruments, San Diego, CA). The test apparatus contained two chambers, each 21 × 25.5 × 16.5 (height) cm. Subjects were placed in the lighted chamber and allowed up to 4 min to enter the darkened chamber, whereupon the door closed and they received a mild foot shock (2 mA, 2 s); animals failing to enter voluntarily were forced into the dark chamber after 4 min and then shocked. Twenty-four hours later, the animals were tested in the apparatus to determine whether they would cross into the dark chamber.

The sequence of behavioral tests was chosen so as to avoid carryover from one test to another, with the last test as the only one involving a pretraining session and administration of shocks. The validity of this sequence was verified by preliminary experiments with unoperated animals.

**Tissue Preparations.** The day after completion of behavioral testing, animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and blood was collected by cardiac puncture using syringes containing 3.8% sodium citrate (pH 7.4) as an anticoagulant, with the volume adjusted to achieve a final concentration of 0.38% after blood collection. As described previously (Moret and Briley, 1991; Slotkin et al., 1991) platelet-rich plasma was isolated by serial centrifugation at 100g (twice), 250g, and 600g, after which all the supernatant fractions were pooled, diluted 50% with calcium-free Krebs-Henseleit medium, and sedimented at 39,000g. The presence of viable platelets was verified under a microscope and platelet membranes were prepared (Moret and Briley, 1991; Slotkin et al., 1991). The membrane suspension was divided into several aliquots that were flash-frozen and stored at −45°C until used.

Brains were dissected to obtain the frontal/parietal cortex, temporal/occipital cortex, hippocampus, corpus striatum, midbrain, brainstem, and cerebellum (including flocculi). In the unoperated and 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 use.

**[^3H]Paroxetine Binding.** The cell membrane fraction was prepared from brain regions by techniques described previously (Moret and Briley, 1991). The suspension was then used immediately for[^3H]paroxetine binding (Moret and Briley, 1991), using approximately 10 μg (platelets) or 100 μg (brain regions) of membrane...
protein and paroxetine[phenyl-6-3H] (specific activity 25.4 Ci/mmol, New England Nuclear Corp., Boston, MA) with or without addition of 100 μM serotonin (Sigma Chemical Co., St. Louis, MO) to displace specific binding. Incubations lasted 120 min at 20°C, and were stopped by addition of 5 ml of ice-cold buffer, followed by vacuum filtration and washing onto Whatman GF/C filters presoaked in 0.05% polyethyleneimine (Sigma). Nonspecific binding was approximately 5% of the total.

Because of the large number of tissues involved in this study, four treatment groups × 3 tissues × 10 animals per group (a total of over 100 membrane preparations to be analyzed simultaneously), it was not practicable to run Scatchard analyses on each individual preparation. Accordingly, the following strategy was adopted. We examined binding at a single ligand concentration in preparations from every animal, using 85 pM [3H]paroxetine, a concentration above the reported K_d (Moret and Briley, 1991), but nevertheless below full saturation of the binding site. The strategy of using a single ligand concentration enables the detection of drug- or age-induced changes but does not permit distinction of whether the changes are in K_d or B_max. Accordingly, membranes from several animals within a given treatment cohort and tissue were then combined to make two additional preparations to be used for Scatchard analyses, which were conducted over a full range of subsaturating to saturating ligand concentrations to identify whether binding alterations resulted from altered K_d or B_max. Duplicate determinations were made of total and nonspecific binding at every ligand concentration for each preparation. Again, studies of platelet and brain membrane preparations were always done simultaneously.

Serotonin Transporter mRNA. Determinations of the mRNA encoding the serotonin transporter were conducted in the midbrain and brainstem, the regions containing the highest concentration of the cell bodies that supply ascending and descending serotonergic innervation (Meister et al., 1995; Fumagalli et al., 1996). Total RNA was isolated by the CsCl method and transporter mRNA was evaluated with Northern blots (Fumagalli et al., 1996). All lanes were loaded with approximately equivalent amounts (20 μg) of total RNA, as confirmed by absorbance readings at 260 nm and by the intensity of ribosomal RNA bands. Results were taken only from undegraded samples having a ratio of 28S:18S ribosomal RNA ethidium bromide staining of 2 and for which little or no DNA contamination was present (as demonstrated by the absence of residual ethidium bromide fluorescence at the origin well). Probe hybridization was carried out as described previously, using a cRNA probe (Fumagalli et al., 1996). Antisense probe was obtained by in vitro transcription with T3 polymerase using [32P]CTP as labeled nucleotide. Blots were quantitated by phosphorimaging (Molecular Dynamics, Sunnyvale, CA; ImageQuant 3.3 software) and were standardized by evaluating the β-actin mRNA band (Fumagalli et al., 1996).

β-Adrenergic Receptors and Adenylyl Cyclase. Cell membrane fractions were isolated, and receptor binding capabilities as-
passively as described above for [3H]paroxetine binding. Single ligand was incubated with the tissue membrane preparation (0.2 mg of membrane protein). Cyclic AMP was analyzed with radioimmunoassay kits (Amersham Corp., Chicago, IL). Enzyme activity was evaluated under several different conditions. First, basal activity was evaluated in the absence of GTP. Second, to determine the dependence of basal activity on participation of G proteins, activity was measured with the addition of 10 μM GTP (Sigma). Third, the maximal G protein-linked response was evaluated in samples containing both GTP and 10 mM NaF. Fourth, maximal total activity of the adenylyl cyclase catalytic unit, independent of receptors or G proteins, was evaluated with 100 μM forskolin (Sigma) + 10 mM MnCl₂ in the presence of GTP. Finally, neurotransmitter receptor-mediated effects were evaluated with either 100 μM isoproterenol or dopamine in the presence of GTP. The concentrations of all the agents used here have been found previously to be optimal for effects on adenylyl cyclase and were confirmed in preliminary experiments (Slotkin et al., 1996).

Data Analysis. Parametric data are presented as means and S.E.s, with intergroup comparisons by multivariate ANOVA (data log-transformed whenever variance was heterogeneous). The factors included age (young versus aged rats), treatment (sham-operated versus OBX, or, in some cases, unoperated versus sham-operated), and brain region (a repeated measure, because more than one region was examined from each animal for each variable). In each case, whenever a significant interaction of OBX × age was found, Fisher’s Protected Least Significant Difference was used to identify specific intergroup differences; posthoc analysis was not undertaken when only main effects of treatment or age were detected without an interaction. Main effects were considered significant at p < .05 and interaction terms at p < .1.

Determinations of adenylyl cyclase activity involved four variables (treatment, age, region, in vitro condition of measurement) and was thus subjected to a four factor ANOVA with two repeated measures (region, condition). Because of significant interactions of age and treatment with the other variables, results were analyzed separately for each region and for each condition within each region. For simplicity, the effects of OBX are given as the percentage change from values in the age-matched, sham-operated groups, but the statistical analyses were conducted on the unmanipulated data.

Intergroup differences for nonparametric categorizations (reactivity, passive avoidance) were evaluated by χ². Scatchard data are given as means and bivariate S.E.s, since both abscissa and ordinate contain dependent variable terms (amount of ligand bound). Differences in transporter or receptor number and affinity were determined by linear regression and analysis of covariance (ANCOVA).

Results

In the first 24 h after surgery, young sham-operated rats showed a small decline in body weight before resuming normal growth (Fig. 1). The young OBX group lost a small additional percentage (4%) but nevertheless resumed a nearly-normal growth rate. Other than the initial postoperative drop, the sham-operated group showed no significant weight differences from unoperated controls (n = 7, data not shown). Aged rats subjected to the sham operation showed the same proportional postoperative decline in body weight as had the younger cohort but recovery to baseline levels took longer. The OBX procedure in the aged animals produced a more persistent and pronounced fall in body weight (11%), although by the time of behavioral testing, the differences from the sham-operated group were no longer statistically significant.

Age-dependent differences were also apparent in the effect of OBX on brain region weights (Fig. 2). In young animals, the lesion produced a small, but significant deficit in only one brain region (frontal/parietal cortex). In aged animals, OBX produced a significantly larger loss of frontal/parietal cortical weight (OBX × age, p < .02) and in addition, decrements were seen in the midbrain, which was unaffected by OBX in young animals (OBX × age, p < .02). Brain region weights in
sham-operated animals were indistinguishable from those of unoperated controls (data not shown).

Behavior. In young animals, reactivity to an air puff, characterized by a sustained postural freeze, was significantly obtunded by OBX (Fig. 3). Aging alone had no effect on the response, as the sham-operated young animals and corresponding aged group had nearly identical responses. However, when aged animals were subjected to the OBX procedure, they did not show the loss of reactivity, and in fact, the tendency was toward increased reactivity. The two age groups also showed major differences in their propensity to novelty-suppressed feeding. Young animals showed no decrease in chocolate milk consumption after OBX but the same procedure caused a significant decrement in the aged animals. In neither age group did OBX change the consumption of water (range of 0–3 ml, data not shown). In the sham-operated groups, the larger, aged rats consumed more chocolate milk than did young rats.

Increased open-field activity is a sine qua non of the OBX lesion (Leonard and Tuite, 1981; van Riezen and Leonard, 1990; Kelly et al., 1997); it is therefore not surprising that both young and aged OBX animals showed increases in both ambulatory and rearing activities (Fig. 4). However, the effect of OBX on ambulation was significantly greater in aged animals than in young animals (significant interaction of OBX x age), an effect in the opposite direction from that associated with aging alone. Similarly, OBX had little effect on grooming behavior in young animals but reduced this activity by 50% in aged animals. Both groups showed an equivalent OBX-induced increase in defecation during open-field testing. We also compared unoperated to sham-operated animals for open-field behavior and found no significant differences (data not shown).

In the passive avoidance testing apparatus, aged animals displayed a longer training latency than did young animals (Fig. 5). Lesioning did not affect the training time in either the young or aged cohorts, indicating no inherent impairment of the motor functions necessary to cross into the dark chamber. Regardless of age, nearly all the sham-operated animals learned the passive avoidance task, as evidenced by

Fig. 6. Effects of OBX on [3H]paroxetine binding to the serotonin transporter in membranes prepared from frontal/parietal cortex of young and aged rats. In the upper left panel, determinations were made in 10 animals from each group, using a single ligand concentration. Data represent means and S.E.s, with comparisons by ANOVA; asterisks denote significant differences between the OBX animals and the corresponding sham group; in addition, the aged group had significantly higher values than the young animals (main effect of age). The remaining panels display Scatchard plots obtained from two additional membrane preparations in each group, with each point representing means and bivariate S.E.s across the two preparations. ANCOVA comparisons for each pair of Scatchard plots are shown within the panels, indicating significant differences in total binding (main effect) but not in slopes. Across all four groups, ANCOVA indicates a significant main effect of OBX (p < .005), a significant main effect of age (p < .0001) and a significant difference in the effect of OBX in young versus aged animals (interaction of OBX x age, p < .0001), again without differences in slope.
no (young) or few (aged) animals crossing into the dark chamber during the subsequent post-training test period. As described previously (Leonard and Tuite, 1981; van Riezen and Leonard, 1990; Kelly et al., 1997), young OBX animals showed severe impairment of passive avoidance learning, with nearly 50% of the lesioned animals crossing. In contrast, aged OBX animals performed this task no differently from the sham-operated group.

**Biochemistry.** Measurements of $[^3]$H]paroxetine binding to the high-affinity, presynaptic serotonin transporter indicated significant age- and lesion-related differences in the frontal/parietal cortex (Fig. 6). At a single ligand concentration, values were increased in young OBX animals relative to sham-operated animals. In contrast, lesioning in aged animals produced a decrease, not an increase, in transporter binding. As found previously (Slotkin et al., 1997b), values for aged controls were higher than in the corresponding group of young animals. To explore the mechanisms underlying these effects on the transporter, Scatchard determinations were undertaken. Sham-operated, aged animals displayed higher binding maxima (abscissa intercept) than in young animals, without a change in transporter affinity (slope). Similarly, the increase in transporter binding seen with OBX in young animals, and the decrease with OBX seen in aged animals, reflected changes in $B_{\text{max}}$ and not $K_d$. In the hippocampus (Fig. 7), there were no significant differences detected at a single ligand concentration but the more sensitive Scatchard determinations verified the presence of a greater effect in the cortex: OBX $\times$ age $\times$ region, $p < .005$. The remaining panels display Scatchard plots obtained from two additional membrane preparations in each group, with each point representing means and bivariate S.E.s across the two preparations. ANCOVA comparisons for each pair of Scatchard plots are shown within the panels, indicating significant differences in total binding (main effect) but not in slopes. Across all four groups, ANCOVA indicates a significant difference in the effect of OBX in young versus aged animals (interaction of OBX $\times$ age, $p < .0001$), again without differences in slope. ANCOVA for comparison of effects in hippocampus versus frontal/parietal cortex confirms the presence of a greater effect in the cortex: OBX $\times$ age $\times$ region, $p < .03$; OBX $\times$ age $\times$ region, $p < .09$.

![Fig. 7. Effects of OBX on $[^3]$H]paroxetine binding to the serotonin transporter in membranes prepared from hippocampus of young and aged rats. Top left: determinations were made in 10 animals from each group, using a single ligand concentration. Data represent means and S.E.s, with comparisons by ANOVA; the lack of significant differences in the hippocampus was distinguishable from the differences found in the frontal/parietal cortex (OBX $\times$ age $\times$ region, $p < .005$). The remaining panels display Scatchard plots obtained from two additional membrane preparations in each group, with each point representing means and bivariate S.E.s across the two preparations. ANCOVA comparisons for each pair of Scatchard plots are shown within the panels, indicating significant differences in total binding (main effect) but not in slopes. Across all four groups, ANCOVA indicates a significant difference in the effect of OBX in young versus aged animals (interaction of OBX $\times$ age, $p < .0001$), again without differences in slope. ANCOVA for comparison of effects in hippocampus versus frontal/parietal cortex confirms the presence of a greater effect in the cortex: OBX $\times$ age $\times$ region, $p < .03$; OBX $\times$ age $\times$ region, $p < .09$.](image-url)
single ligand concentration or with Scatchard determinations (Fig. 8), although the direction of change (decrease) was the same as that seen for OBX effects on the serotonin transporter in the CNS of the young animals.

To determine if the effects of aging and OBX on serotonin transporter binding in brain regions reflected alterations in the level of gene transcription, we determined the effects on the mRNA encoding the transporter (Fig. 9); measurements were made in the midbrain and brainstem, the regions containing the highest concentration of cell bodies for serotonergic projections ascending into the brain (midbrain) or descending into the spinal cord (brainstem). Despite the fact that aged, sham-operated animals exhibited a higher concentration of serotonin transporter sites than did young animals, as determined with [3H]paroxetine binding, transporter mRNA transcript levels were not distinguishable in the two age groups. Indeed, if anything, the levels were lower in aged midbrain compared to that in the young cohort. The results for transporter midbrain mRNA in aged control animals replicate those we reported previously (Fumagalli et al., 1996).

Fig. 8. Effects of OBX on [3H]paroxetine binding to the serotonin transporter in platelet membranes prepared from young and aged rats. Top: determinations were made in 10 animals from each group, using a single ligand concentration. Data represent means and S.E.s, with comparisons by ANOVA; there was no effect of OBX but values in the aged group were higher overall (main effect). Bottom: Scatchard plots obtained from two additional membrane preparations in each group, with each point representing the mean value from the two preparations; S.E.s have been omitted for clarity, but were comparable in magnitude to those seen in the Scatchard plots for brain regions. ANCOVA comparisons across all four groups appear within the panel, again demonstrating only a main effect of age but not lesioning, without changes in slope.

Alterations in transcription also could not account for the effects of OBX, as neither the young nor aged animals showed any significant effects of lesioning on transporter mRNA.

In aged control rats, both the temporal/occipital cortex and the cerebellum displayed significant deficits in β-adrenergic receptor binding capabilities as compared to younger animals (Fig. 10). Scatchard analyses confirmed that the differences seen at a single ligand concentration represented a reduction in the number of receptors, without a change in affinity. The OBX lesion did not affect the number of receptors and did not alter the age-related difference in binding values. Irrespective of age or lesioning, there were significant differences in the slopes of the Scatchard plots between the two brain regions (p < .0001), reflecting the different receptor subtypes predominating in each (Pittman et al., 1980; Minneman et al., 1981; Lorton et al., 1988).

Previous work has shown that aging can affect β-receptor signal transduction downstream from the receptors themselves (Heinsimer and Lefkowitz, 1985; Scarpace et al., 1991; Slotkin et al., 1996). Accordingly, we compared adenyl cyclase activities for measures involving receptor activation as well as postreceptor stimuli. In sham-operated animals, the aged cohort showed deficits in basal adenyl cyclase activity...
in all regions studied: temporal/occipital cortex, cerebellum, and corpus striatum (Table 1). When G protein interactions were enabled, the deficits resolved partially (addition of GTP) or completely (GTP and fluoride). In one region (cerebellum), total cyclase activity was significantly elevated in aged animals, as indicated by an increase in forskolin-Mn$^{2+}$-stimulated activity relative to that seen in young animals. Finally, we compared the effects of the catecholamine receptor agonists, isoproterenol and dopamine, across the two age groups, measured in the presence of GTP to enable receptors to interact with G proteins; because of the inherent age-dependent differences in enzyme activity, stimulation was calculated as the percent increase over values obtained with GTP alone. None of the regions showed significant effects of aging on the receptor-mediated response, regardless of whether the stimulant was a β-receptor agonist (isoproterenol in temporal/occipital cortex and cerebellum) or dopamine (striatum).

Nevertheless, marked differences in adenylyl cyclase signaling emerged when young and aged animals were subjected to OBX (Fig. 11). In the young group, lesioning produced marked elevations of forskolin-Mn$^{2+}$-stimulated activity in every region and an equally robust increase in fluoride stimulation in the cerebellum. Consequently, catecholamine receptor-mediated stimulation, whether by isoproterenol or dopamine, was also enhanced in the young OBX group relative to its sham-operated control. In contrast, aged animals subjected to the OBX procedure failed to show any increases in forskolin-Mn$^{2+}$-stimulated or fluoride-stimulated cyclase activity and instead tended to show decreases in the cerebellum and striatum. Similarly, in the aged OBX animals, receptor-mediated activity, rather than being enhanced as seen in the young OBX group, was unaffected (temporal/occipital cortex, striatum) or decreased (cerebellum).

**Discussion**

The present results indicate that the response to OBX differs between young and aged animals at structural, behavioral, and cellular levels. In the aged group, the lesion produced a greater tissue loss in the frontal/parietal cortex than that seen with young animals and, in addition, weight defi-
Effects of OBX Bullectomy on Adenylyl Cyclase

Table 1: Adenylyl cyclase activity in sham-operated animals

<table>
<thead>
<tr>
<th>Region and Measure</th>
<th>Young</th>
<th>Aged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temporal/Occipital Cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal (pmol/mg protein/min)</td>
<td>68 ± 4</td>
<td>49 ± 3*</td>
</tr>
<tr>
<td>+GTP</td>
<td>127 ± 8</td>
<td>103 ± 6*</td>
</tr>
<tr>
<td>+GTP + Fluoride</td>
<td>150 ± 12</td>
<td>150 ± 9</td>
</tr>
<tr>
<td>+GTP + Forskolin + Mn²⁺</td>
<td>6506 ± 665</td>
<td>7820 ± 512</td>
</tr>
<tr>
<td>% Stimulation by Isoproterenol</td>
<td>15 ± 2</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal (pmol/mg protein/min)</td>
<td>97 ± 5</td>
<td>68 ± 4*</td>
</tr>
<tr>
<td>+GTP</td>
<td>166 ± 9</td>
<td>144 ± 7</td>
</tr>
<tr>
<td>+GTP + Fluoride</td>
<td>249 ± 18</td>
<td>285 ± 18</td>
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<tr>
<td>+GTP + Forskolin + Mn²⁺</td>
<td>4447 ± 403</td>
<td>7088 ± 514*</td>
</tr>
<tr>
<td>% Stimulation by Isoproterenol</td>
<td>119 ± 15</td>
<td>142 ± 17</td>
</tr>
<tr>
<td>Striatum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal (pmol/mg protein/min)</td>
<td>42 ± 1</td>
<td>31 ± 1*</td>
</tr>
<tr>
<td>+GTP</td>
<td>71 ± 4</td>
<td>62 ± 3*</td>
</tr>
<tr>
<td>+GTP + Fluoride</td>
<td>135 ± 12</td>
<td>109 ± 9</td>
</tr>
<tr>
<td>+GTP + Forskolin + Mn²⁺</td>
<td>5320 ± 507</td>
<td>6994 ± 361</td>
</tr>
<tr>
<td>% Stimulation by Dopamine</td>
<td>40 ± 9</td>
<td>47 ± 5</td>
</tr>
</tbody>
</table>

Data represent means and standard errors obtained from 10 animals in each group. Stimulation by isoproterenol or dopamine represents the percentage increase over those obtained with GTP alone. Across all three regions, ANOVA indicates significant age-related differences that depend on the type of cyclase measurement (age × measure interaction, p < .0001) and the interaction is also present (p < .0001) in each region taken individually. Asterisks denote individual measures that differ between young and aged cohorts.

Serotonergic and catecholaminergic pathways represent prominent features of projections to and from the olfactory bulbs and pharmacological targeting of these transmitter systems reverses the behavioral effects of OBX (Leonard and Tuite, 1981; Kelly et al., 1997). Accordingly, we determined whether the differential age effects on behavior were associated with corresponding alterations in monoaminergic systems. In the frontal/parietal cortex, OBX in young animals produced an increase in the number of presynaptic serotonin transporter sites, as evidenced by an enhanced capacity to bind [³H]paroxetine. Although aging, by itself, also increased the number of sites, the OBX lesion in aged animals produced a change in the opposite direction, namely a decrease. A similar pattern emerged for the interaction of aging with OBX in the hippocampus, albeit with a smaller magnitude of effect when compared to the frontal/parietal cortex: OBX

![Fig. 11. Effects of OBX on adenylyl cyclase activity in brain regions of young and aged rats, presented as the percent change from values in the corresponding sham-operated animals. Data represent means and S.E.s obtained from 8 to 10 animals in each group. ANOVA across all measures appears within each panel and asterisks denote individual values for which the effect of OBX in the aged rats differs from that seen for comparably lesioned young rats. Across all three regions, the effect of OBX in aged rats can be distinguished from that in young rats (OBX × age, p < .005). The OBX effect differs according to the in vitro conditions used in the cyclase measurement (OBX × age × measure, p < .0001; OBX × age × measure × region, p < .0001) and differs among the regions (OBX × age × measure × region, p < .0001). Values for sham-operated animals appear in Table 1.](image-url)
increased transporter expression in young animals but decreased it in aged animals. Although the two regions share the same effect on serotonin transporter sites, the hippocampus did not show any weight loss after OBX, and it is therefore unlikely that the age dependence of the effects of lesioning on serotonergic function is secondary to atrophy. Indeed, degenerative changes have been shown to produce an increase in transporter gene transcription (Meister et al., 1995), whereas we saw no change in transporter mRNA. The lack of change of mRNA also indicates that changes in transporter number probably reflect post-transcriptional alterations related to cellular function. In support of the concept of age-specific CNS effects, blood platelets from young and aged OBX animals showed only small changes that were in the same direction (decrease), regardless of age, the same type of change reported previously for young animals (Leonard and Tuite, 1981; Kelly et al., 1997). Thus, in parallel with behavioral differences in the effect of OBX, actions directed toward the presynaptic serotonin transporter show opposite changes in brain regions of aged versus young animals.

Earlier work in human geriatric depression indicates a dichotomy between platelet serotonin transporter expression and function (Nemeroff et al., 1988; Slotkin et al., 1989, 1997a). Specifically, elderly depressed patients with normal dexamethasone suppression tests show decreased serotonin uptake and reduced imipramine effect, whereas those with non-suppression do not (Slotkin et al., 1997a). Both groups, however, show decreased transporter expression as monitored by [3H]imipramine binding (Nemeroff et al., 1988; Slotkin et al., 1997a). Accordingly, it would be useful to examine whether similar divergence of transporter expression and function occur with the OBX model and specifically whether the dichotomy is selective for age or suppression status. This was not done in the current study because the preparation of platelet membranes for binding studies is not compatible with the intact platelets needed for serotonin uptake measurements. However, previous work on platelet transporter function in aged rats with or without dexamethasone treatment indicates poor homology between effects on platelet transporter expression or function and those on the CNS transporter (Slotkin et al., 1997b). Accordingly, platelet studies in the OBX model of geriatric depression may be an inappropriate representation of corresponding events in the CNS and might be specifically different from platelet effects seen in human depression.

Among the most prominent neurotransmitter-related effects of aging are the loss of adrenergic receptors and of cell-signaling properties required by those receptors (Makman et al., 1978, 1980; Heinsimer and Lefkowitz, 1985; Slotkin et al., 1996). In the current study, aged sham-operated animals displayed lower basal adenylyl cyclase activity and reduced β-adrenergic receptor numbers in temporal/occipital cortex and cerebellum. However, in each case, the receptor linkages for catecholamines (assessed with isoproterenol in the cortex and cerebellum, or with dopamine in the striatum) to stimulate adenylyl cyclase showed no specific decrement: activities in the presence of stimulant were affected only to the same extent as was activity without the neurotransmitter stimulant (i.e., no change in the percent stimulation evoked by neurotransmitter agonists). However, upon OBX lesioning, major differences appeared in the neurotransmitter response of aged animals as compared to the young control. OBX in young animals elicited up-regulation of adenylyl cyclase total activity (increased forskolin-Mn2+ response) and of the linkage of cyclase to activation of G-proteins (fluoride response); accordingly, there was an increase in the G protein-linked response to activation of β-adrenergic (isoproterenol) or dopaminergic receptors. In aged animals, the same lesion produced either no change or an actual loss of responsiveness. The age-dependent differences were not secondary to altered receptor expression, which was unaffected by OBX at either age. Thus, as was true for the serotonergic system, catecholaminergic pathways show major, even opposite, age-related differences in their response to OBX.

Our findings thus substantiate the idea that, in animals, lesions that elicit many of the features of major depressive disorder in human, show distinctly different and even opposite response profiles in aged brain, effects that are expressed at both behavioral and neurochemical levels. Equally important, the neurotransmitter systems displaying age-specific effects are those involved in the mechanisms thought to underlie depression in human, and that represent the systems targeted by the major antidepressants. Accordingly, the OBX model may provide a useful paradigm with which to identify age-dependent differences in synaptic adaptability that may underlie the specificity of biological markers for depression or the effectiveness of antidepressants. This model may then prove useful in uncovering additional biological markers that can distinguish subpopulations that do or do not respond to drug therapies (Kin et al., 1997; Slotkin et al., 1997a).

References


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