Olfactory bulbectomy attenuates cardiovascular sympathoexcitatory reflexes in rats

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Moffitt, Julia A., Angela J. Grippo, Philip V. Holmes, and Alan Kim Johnson. Olfactory bulbectomy attenuates cardiovascular sympathoexcitatory reflexes in rats. Am J Physiol Heart Circ Physiol 283: H2575–H2583, 2002.—Bilateral removal of the olfactory lobes in rats produces a number of behavioral, endocrine, and neurochemical alterations in the brain. Little is known, however, regarding the effects of this treatment on cardiovascular function and autonomic reflexes. Male Sprague-Dawley rats underwent bilateral surgical ablation of the olfactory bulbs (n = 10) or were sham operated (n = 8). After 3 wk of recovery, animals were instrumented with femoral catheters and a lumbar sympathetic nerve recording electrode. After 24 h of recovery, cardiovascular responses to arterial baroreflex manipulation, air jet stress, and smoke exposure were recorded. Olfactory bulbectomized rats demonstrated attenuated sympathoexcitatory responses to hypotension, air jet stress, and smoke exposure, as well as elevated basal blood pressure, compared with sham-operated rats. These data indicate that the integrity of the olfactory bulbs in rats is important for the elicitation of normal cardiovascular and autonomic responses to a number of evocative stimuli.

arterial baroreflex; lumbar sympathetic nervous system activity; air jet stress; smoke exposure; autonomic nervous system; heart rate; arterial blood pressure

THE OLFACTORY BULBS constitute 4% of the mass of the rat brain (3). Rats rely heavily on olfaction to assess the environment and recognize predators and food sources. The olfactory bulbs have extensive connections with several parts of the forebrain and midbrain (for reviews, see Refs. 11 and 15), and it is not surprising that lesions in these forebrain structures result in behavioral, neurochemical, and physiological changes. For example, bilateral removal of the olfactory lobes (olfactory bulbectomy) in rats produces increased exploratory behavior (28), hyperactivity (7), and reduced sexual behavior (16). Endocrine changes, such as altered circadian rhythms of corticosterone and body temperature, are also present in olfactory bulbectomized (OBX) rats (18), and immune alterations such as changes in lymphocytes and neutrophils, among others, have been reported in OBX rats (27). These changes in functional responses are likely to be associated with one or more of the neurochemical alterations observed after olfactory bulbectomY, including changes in serotonin, norepinephrine, acetylcholine, GABA, and glutamate (for a review, see Ref. 11).

Although the neurobiological effects of olfactory bulbectomy have been studied extensively, little is known regarding the consequences of this treatment on cardiovascular function and autonomic reflexes. Olfactory stimuli have been shown to produce autonomic and cardiovascular changes in rats. For example, in 1929, Allen (1) observed significant alterations in respiration and blood pressure in response to various olfactory stimuli in awake, anesthetized, and sleeping humans (3). In conscious rats, smoke exposure elicits a slight pressor response, bradycardia, and an increase in sympathetic nervous system activity (21). The neural substrate for olfactory stimuli-induced autonomic responses in rats appears to be a multisynaptic pathway from the olfactory bulbs to the dorsal medulla, specifically to the nucleus tractus solitarius (NTS). This olfactory bulb-NTS pathway is conducted via a relay in the ventral taenia tectae and central nucleus of the amygdala (4). The NTS is the primary site for integration of many visceral afferent inputs to the central nervous system, including those arising from peripheral baroreceptors (25). Because of the connections among olfactory nuclei and cardiovascular control regions in the central nervous system, it is of interest to determine whether altered autonomic responses are a characteristic of olfactory bulb lesions.

An interesting reason for examining autonomic responses in OBX rats is that this procedure is used as an experimental model of psychological depression. The OBX model has reasonable predictive validity and has been used to screen for pharmacological antidepressant treatments including tricyclic antidepressants, serotonin reuptake inhibitors, and atypical antidepressants (30). Depression can influence the pathogenesis

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of cardiovascular disease (2), perhaps through changes in autonomic regulation. For instance, patients with major depressive disorder display altered cardiovascular parameters such as increased resting heart rate (HR) (14) and reduced HR variability (13) as well as elevated components of the sympathetic nervous system and altered baroreflex sensitivity (17, 29). Similar changes have been observed in an animal model of depression (9). Thus examination of autonomic regulation in OBX rats may provide insight regarding the role of autonomic mechanisms underlying cardiovascular regulation and mood changes.

The purpose of the present study was to investigate cardiovascular and autonomic function in OBX rats. We hypothesized that due to the heavy reliance on olfaction for normal behavior in rats, removal of the olfactory lobes would produce an attenuation of cardiovascular autonomic reflexes. We assessed evoked changes in arterial pressure, HR, and sympathetic nerve activity (SNA) in response to arterial baroreceptor manipulation, air jet stress, and smoke exposure in conscious, unrestrained rats subjected to bilateral olfactory lobe ablation. Evaluating the autonomic responses to each of these manipulations allowed us to determine whether changes in cardiovascular and autonomic regulation were specific to arterial baroreflex function or more generalized in nature.

METHODS

Animals. Male Sprague-Dawley rats (n = 18) obtained from Harlan (Indianapolis, IN) were used for all experimental procedures. Animals were housed singly in environmentally controlled conditions and maintained on a 12:12-h light-dark cycle and given food and water ad libitum. Animals were 6–7 wk of age at the beginning of the experiments. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees of the University of Georgia and University of Iowa.

Bulbectomy. Bilateral ablation of the olfactory bulbs was performed using methods similar to those described previously (10). At least 1 wk after arrival at the University of Georgia animal facilities, rats were randomly divided into bilateral OBX (n = 10) and sham-operated (sham; n = 8) groups. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (Nembutal; Abbott Laboratories; Chicago, IL) mixed with ketamine HCl (40 mg/kg, Ketaset; Fort Dodge, IA).

After anesthesia, the rats underwent aseptic surgical preparation and were placed in a stereotaxic apparatus. With the use of a midline incision, two 2-mm-diameter burr holes were drilled 6 mm rostral to the bregma and 1 mm to the right and left of the midline. With the use of an aspirator, the olfactory bulbs were removed through a 2-mm-diameter plastic pipette tip, using special care to avoid damage to the frontal cortex. The cavity was then filled with Gelfoam (Upjohn; Kalamazoo, MI) to minimize bleeding. The incision sites were sutured, and the animals were given 2–3 ml of saline and a subcutaneous injection of Banamine (1 mg/kg, Elkins-Sinn; Cherry Hill, NJ) for postoperative pain. Sham animals were treated identically, with the exception that the olfactory bulbs were not removed. All rats survived the surgery. After at least 14 days of recovery from surgery, animals were shipped to animal care facilities within the Department of Psychology at the University of Iowa and allowed an additional week to adapt to the animal colony before any further experimental manipulations took place.

Catheter and electrode surgical procedures. Surgical procedures for the placement of femoral catheters and lumbar sympathetic recording electrodes were conducted while the animals were under halothane anesthesia, using aseptic surgical techniques. Polyethylene (polyethylene-10 fused to polyethylene-50) catheters were inserted into the abdominal aorta and vena cava via the left femoral artery and vein for measurement of arterial pressure and administration of vasodilatory drugs, respectively.

With the use of a midline abdominal incision, a branch of the lumbar sympathetic chain was dissected free. A bipolar Teflon insulated silver wire electrode (Medwire, 0.005 in. diameter, 36 gauge) threaded through silastic tubing (0.025 in. inner diameter (ID)) was placed around the isolated nerve. The nerve-electrode complex was covered with polyvinylsiloxane gel (Coltene President; Mahwah, NJ), which was allowed to harden before closure. A ground wire was sewn to the abdominal wall, and a subcutaneous wound was closed. The catheters and the wire of the lumbar sympathetic recording electrode were tunneled subcutaneously and exteriorized to the dorsal cervical region. Catheters were filled with 10 U/ml heparinized saline and capped with an airtight plug until the experiment. Animals were given subcutaneous fluids and Stadol (3 mg/kg, Bristol Myers Squibb; Princeton, NJ) for postoperative analgesia. After immediate recovery from the anesthesia, animals were returned to their cages for 24 h of additional recovery.

Experimental procedures. Unrestrained animals were placed in a plastic experimental cage (20 × 24 × 16 cm) lined with bedding. This cage was then placed inside a Faraday cage to reduce electrical noise. The arterial catheter was connected to a transducer for the recording of arterial pressure. Mean arterial pressure (MAP) was derived electronically using a low-pass filter. HR was determined by measuring the number of heartbeats triggered from the arterial pressure pulse. Lumbar sympathetic nerve activity (LSNA) was amplified 2,000 times using a Grass preamplifier (P511) and filtered using a high-pass frequency level of 30 Hz and a low-pass frequency level of 3 kHz. Action potentials were monitored using a Tektronix oscilloscope and a custom-made audio monitor (Bioengineering, University of Iowa). Nerve activity was rectified and integrated using a RMS converter with a time constant of 28 ms. The rectified, integrated signal was then electronically averaged, and this mean signal was used as the relative measure of LSNA. Background noise was determined at the end of the experiment, when SNA was eliminated by an intravenous bolus dose of the ganglionic-blocking agent chlorisondamine (5 mg/kg). This level of background noise was then subtracted from the recorded LSNA before calculation of the percent baseline values and baroreflex sigmoid curve fitting. In some animals (sham, n = 5; OBX, n = 6), the change in blood pressure in response to ganglionic blockade was recorded and analyzed. Data signals were gathered and analyzed with a PowerLab (ADInstruments) data acquisition system.

Baroreflex assessment protocol. Baseline hemodynamic parameters were recorded for 20–40 min before experimental manipulations to ensure stabilization of MAP, HR, and LSNA. After the collection of baseline parameters, arterial baroreflex curves were generated by producing ramp changes in arterial pressure over −2–3 min. Initially, MAP was increased to 170–180 mmHg by infusion of the α1-adrenergic receptor agonist phenylephrine (PE) at increasing rates (2–
25 μg·kg⁻¹·min⁻¹). MAP, HR, and LSNAs were allowed to return to within 10% of baseline values before we proceeded with the experimental protocol. Arterial pressure was then decreased to 50–60 mmHg within 2–3 min by infusion of the vasodilator sodium nitroprusside (SNP) at sequentially increasing rates (10–100 μg·kg⁻¹·min⁻¹). The rate of change of arterial pressure was held constant by observing the recorded pressure alteration and varying the rate of infusion to produce a smooth ramp of pressure increase or decrease. Care was taken to keep the rate of change of arterial pressure similar in all animals, at ~1–2 mmHg/s. Volumes infused did not exceed 100 μl. Baroreceptors were always activated first (PE infusion) before unloading (SNP infusion) to minimize any potential effects of reflexly released humoral agents, such as vasopressin or angiotensin II, on baroreflex function.

Air jet stress. After the baroreflex assessment, the cardiovascular response to air jet stress was performed in subgroups of sham (n = 4) and OBX (n = 4) rats used in the baroreflex protocol. In this protocol, a flexible hose (0.7 cm ID) connected to a cylinder of compressed room air was directed to the top of the head of the rat from a distance of ~5 cm. Air pressure was maintained at 20 psi, an intensity that was insufficient to part the fur on the rat’s head. The air jet stimulus was directed at the rat for a period not exceeding 3 min. During this time, the changes in MAP, HR, and LSNAs were recorded. Care was taken not to include data in the analysis during animal movement. The average of all stable LSNAs recordings that were free from movement artifacts during this 3-min period were used as the mean LSNAs response during air jet stress. Animals were allowed at least 20–40 min before any further experimental manipulations took place.

Smoke exposure. This experiment was performed in the same subgroups of sham (n = 4) and OBX (n = 4) rats used in the air jet stress and baroreflex protocols to evaluate the effects of bilateral olfactory lobe ablation on the cardiovascular response to smoke exposure. A 20-ml syringe was filled with smoke from a lit cigarette (Virginia Slims, filtered, nonmenthol) by application of negative pressure. The smoke was then ejected through a tube connected to the syringe and directed at the nose of the rat while changes in MAP, HR, and LNSAs were recorded. Any movement by the animal during the response was noted, and data during this period were subsequently removed from analysis. The average of all stable LSNAs recordings that were free from movement artifacts were used as the mean LSNAs response during smoke exposure.

Verification of olfactory bulbectomy. At the end of the experimental protocols, rats were euthanized with an overdose of anesthetic. The brains of the rats were removed, and the completeness of olfactory bulb removal was verified by recording the wet weight of the bulbs in both groups. Consistent with previously reported procedures (10), we established before the experiments that data from OBX rats with recoverable olfactory bulb tissue exceeding 10 mg would be excluded from analysis. In addition, frontal lobes were examined in both groups of rats, and it was established before the experiment that animals with damage to this area were to be excluded from the study.

Data analysis. For baroreflex analysis, HR and LSNAs were determined at different levels of MAP during PE and SNP infusion. Data relating changes in HR or LSNAs to MAP were fit to a sigmoid logistic function (12) using a standard software package (SigmaPlot, Jandel Scientific). The equation used for this mathematical model is as follows

\[
\text{LSNA or HR} = \frac{(P_1 - P_3)}{[1 + \exp(P_2(MAP - P_3))] + P_1}
\]

Parameters (\(P_1\)–\(P_3\)), which are used to describe basic baroreflex function, were generated from data fit to the logistic function. These parameters were \(1\) the maximum LSNAs or HR during decreases in arterial pressure (\(P_1\)); \(2\) the coefficient used to calculate the gain as a function of pressure (\(P_2\)); \(3\) the inflection point or MAP at the midpoint of the curve (\(P_3\)); and \(4\) the minimum LSNAs or HR at an increased arterial pressure (\(P_3\)). In addition, the gain (\(G\)) of the reflex at each level of arterial pressure was calculated for the entire baroreflex curve using the following equation

\[
G_{\text{MAP}} = \frac{(P_1 - P_3)P_2\exp(P_2(G_{\text{MAP}} - P_3))}{1 + \exp(P_2(G_{\text{MAP}} - P_3))}^2
\]

For each individual animal’s fit curve, the four parameters (\(P_1\)–\(P_3\)) and gain were derived. These parameters and the gain of the baroreflex curve were averaged within a group. The average parameters were then statistically compared (sham vs. OBX) using independent Student’s t-tests. The mean parameters and gain were used to generate an average baroreflex curve for each group.

The mean responses in MAP, HR, and LSNAs to air jet stress and smoke exposure were analyzed by two-way ANOVA for repeated measures while changes from baseline were also compared using independent Student’s t-tests. A probability of \(P < 0.05\) was considered statistically significant. All statistical analyses were performed using the SigmaStat (Jandel Scientific) software package for IBM.

Lumbar sympathetic nerve responses to baroreceptor manipulation, air jet stress, and smoke exposure were expressed as a percentage of baseline (control) LSNAs before experimental interventions. Baseline LSNAs was considered to be 100%. This analysis allows for direct evaluation of the animal’s capacity to reflexly increase or decrease LSNAs relative to its basal level.

RESULTS

MAP was moderately, although significantly, elevated in the OBX (125 ± 2 mmHg) compared with sham (117 ± 2 mmHg) animals. There was no significant difference between the groups with respect to baseline HR (sham: 371 ± 6 beats/min vs. OBX: 370 ± 6 beats/min). Body weight was lower in rats that underwent removal of the olfactory bulbs (284 ± 7 g) compared with sham animals (346 ± 18 g). All of the OBX animals in this study had olfactory bulb tissue of <10% of the olfactory bulb wet weight of normal animals. Hence, the recovered olfactory bulb tissue wet weight was significantly lower in the OBX rats (sham: 65.4 ± 8 mg vs. OBX: 5.9 ± 0.7 mg), <10% of the recovered tissue from sham rats. None of the animals sustained damage to the frontal lobes. Therefore, there were no animals eliminated from the studies on the basis of postmortem histological analysis.

Measurement of LSNAs. Raw, amplified LSNAs tracings from sham and OBX rats are illustrated in Fig. 1. The rectified, integrated, and amplified LSNA signal (in mV) was converted to absolute LSNA (in μV) using the following methods: \(1\) calibration of the signal via known microvolt input, \(2\) dividing the amplified signal by the amplification (×2,000), and \(3\) measuring and subtracting out background electrical noise after ganglionic blockade. With the use of these three procedures, absolute microvolt activity was calculated for
Baroreflex control of HR. Sigmoid baroreflex curves demonstrating the goodness of fit for a sham and OBX rat are shown in Fig. 2. Mean sigmoid curves describing the baroreflex control of HR in the sham and OBX groups are depicted in Fig. 3A, whereas Fig. 3B illustrates the gain of the baroreflex curves as a function of arterial pressure. There were no changes in baroreflex control of HR after bilateral olfactory bulbectomy (Fig. 3). This was further evidenced by no significant differences in any of the parameters describing the baroreflex curves (Table 1). In addition, the maximum gain and the gain of the HR baroreflex curves throughout the entire range of MAP were also similar between sham and OBX animals (Fig. 3B and Table 1).

Baroreflex control of LSNA. Sigmoid baroreflex curves demonstrating the goodness of fit for a sham (A) and an OBX (B) rat. Circles represent recorded data points, and the line represents the fit curve. HR, heart rate; MAP, mean arterial pressure.

Fig. 2. Baroreflex curves illustrating goodness of fit for a sham (A) and an OBX (B) rat. Circles represent recorded data points, and the line represents the fit curve. HR, heart rate; MAP, mean arterial pressure.
Table 1. Curve parameters defining baroreflex control of heart rate

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Maximum, beats/min</th>
<th>Midpoint, mmHg</th>
<th>Minimum, beats/min</th>
<th>Peak Gain, beats/min ( \cdot ) mmHg (^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>8</td>
<td>494 ± 15</td>
<td>125 ± 8</td>
<td>242 ± 22</td>
<td>-2.7 ± 0.7</td>
</tr>
<tr>
<td>OBX</td>
<td>10</td>
<td>515 ± 11</td>
<td>121 ± 3</td>
<td>267 ± 9</td>
<td>-2.9 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. OBX, olfactory bullectomized.

Fig. 3. A: mean baroreflex curves describing reflex control of HR. Both sham (n = 8) and OBX (n = 10) groups exhibited a similar HR response to increases and decreases in MAP. B: mean curves illustrating the instantaneous gain of baroreflex control of HR for sham (n = 8) and OBX (n = 10) rats. Both groups exhibited a similar baroreflex gain throughout the range of MAP.

Fig. 4. Baroreflex curves illustrating goodness of fit for a sham (A) and an OBX (B) rat. Circles represent recorded data points, and the line represents the fit curve.

Significantly lower in OBX rats (Fig. 5A and Table 2). Figure 5B illustrates the gain of the baroreflex curve as a function of arterial pressure. The maximum gain (slope at inflection point) was not significantly different between groups (Fig. 5B and Table 2).

Cardiovascular responses to air jet stress. Mean data illustrating the cardiovascular response to air jet stress are shown in Fig. 6. When compressed air was directed at the rats, the animals exhibited an increase in MAP, HR, and LSNA. Movement artifacts in the LSNA recording were noted in the record and were subsequently excluded from the data analysis. Because OBX rats had a slightly but significantly higher basal MAP (sham: 117 ± 2 mmHg vs. OBX: 125 ± 2 mmHg), both absolute responses and changes from baseline are shown. Although the absolute increase in MAP (sham: 136 ± 7.7 mmHg vs. OBX: 132 ± 5.7 mmHg) was not significantly different between the groups, the increase from baseline was significantly attenuated in the OBX rats (sham: 18 ± 1.4 ∆mmHg vs. OBX: 9 ± 3.1 ∆mmHg; Fig. 6). In addition, both the absolute increase in LSNA and the increase in LSNA from baseline (sham: 266 ± 42 ∆%LSNA vs. OBX: 112 ± 16 ∆%LSNA) in response to air jet stress were significantly attenuated after bilateral olfactory bullectomy (Fig. 6). Although there was a trend for a reduced HR response to air jet stress in the OBX rats (sham: 118 ± 26 beats/min vs. OBX: 81 ± 20 ∆beats/min), this effect was not significantly different between groups.

Cardiovascular responses to smoke exposure. Mean values illustrating the cardiovascular response to smoke exposure are shown in Fig. 7. Upon exposure to the smoke stimulus, animals exhibited a pressor, bradycardic, and sympathoexcitatory response. Because OBX rats had a higher basal MAP (sham: 115 ± 3.5 mmHg vs. OBX: 123 ± 8.2 mmHg), both the absolute responses and changes from baseline are shown. There were no significant differences in the absolute change or increase from baseline in MAP (sham: 136 ± 3.8 mmHg vs. OBX: 144 ± 4.6 mmHg) or decrease in HR (sham: 313 ± 24 beats/min vs. OBX: 323 ± 17 beats/min) in response to smoke exposure between the groups. However, the absolute response and increase in LSNA from baseline were significantly attenuated in the OBX group (sham: 437 ± 93 %LSNA vs. OBX: 200 ± 16 %LSNA).

Hemodynamic response to ganglionic blockade. At the end of the experiment, an intravenous bolus injection of chlorisondamine (5 mg/kg) was given to eliminate postganglionic LSNA. The change in blood pressure was recorded and compared between some of
was not significantly different (sham: 66 ± 3 mmHg vs. OBX: 123 ± 3 mmHg). Although the absolute MAP after ganglionic blockade was not significantly different (sham: 66 ± 3 mmHg vs. OBX: 65 ± 2 mmHg), the change from baseline was significantly greater in OBX rats (−58 ± 2 ΔmmHg) versus sham rats (−50 ± 2 ΔmmHg).

**DISCUSSION**

The purpose of the current investigation was to determine the effect of removal of the olfactory bulbs on cardiovascular autonomic responses in conscious, unrestrained rats. Although there have been numerous neurochemical and behavioral studies conducted in OBX rats, cardiovascular reflex responses in this model are not well characterized. To investigate these effects, we assessed changes in HR and LSNA in response to baroreceptor manipulation, air jet stress, and smoke exposure in conscious rats after bilateral olfactory bulb ablation or sham operation. The results indicate that OBX rats have an attenuated maximal increase in LSNA in response to a baroreceptor inhibition. In addition, OBX rats demonstrated an attenuated ability to increase MAP and LSNA from baseline in response to air jet stress as well as a blunted LSNA response to smoke exposure. These outcomes indicate that the integrity of the olfactory bulbs in rats is necessary for the brain to generate normal sympathoexcitatory responses to a number of physiological stimuli.

Cardiovascular responses to baroreceptor inhibition, air jet stress, and smoke produce markedly different hemodynamic responses, although all three of these stimuli elicit a sympathoexcitatory response. Increases in LSNA in rats that underwent bilateral olfactory bulb ablation were significantly attenuated during all three stimuli. In addition, although the maximal increase in response to baroreceptor unloading was attenuated in OBX rats, there was no change in the gain of the arterial baroreflex. These data, taken together, indicate that removal of the olfactory bulbs produces a general attenuation in sympathetic activation rather than eliciting a specific dysfunction of the arterial baroreceptor reflex.

However, it is possible that the olfactory bulbs may influence baseline sympathetic tone rather than modulate sympathoexcitatory responses. Basal MAP was moderately, although significantly, elevated in OBX rats. Blockade of postganglionic sympathetic activity resulted in a greater decrease in MAP in OBX rats. This suggests that there is a greater basal SNA in OBX rats, because elimination of SNA results in a greater decrease in MAP from baseline. We attempted to measure absolute LSNA activity in both groups of rats by 1) calibration of the signal via known microvolt input, 2) dividing the amplified signal by the amplification (×2,000), and 3) measuring and subtracting out background electrical noise. With the use of these three procedures, absolute microvolt activity was calculated for each rat. We did not detect any differences

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**Table 2. Curve parameters describing baroreflex control of LSNA**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Maximum, %LSNA/mmHg</th>
<th>Midpoint, %LSNA/mmHg</th>
<th>Minimum, %LSNA/mmHg</th>
<th>Peak Gain, %LSNA/mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>8</td>
<td>338 ± 31</td>
<td>102 ± 3</td>
<td>45 ± 8</td>
<td>−7.1 ± 1.4</td>
</tr>
<tr>
<td>OBX</td>
<td>10</td>
<td>257 ± 17*</td>
<td>112 ± 2</td>
<td>56 ± 5</td>
<td>−5.3 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. LSNA, lumbar sympathetic nerve activity. *P < 0.01 from the sham group.
between groups with respect to absolute baseline LSNA (sham: 0.91 ± 0.2 μV·s vs. OBX: 0.96 ± 0.2 μV·s). Although we attempted to standardize the surgical implantation of the LSNA recording electrodes, it is possible that a high degree of variability was introduced using this method and therefore prevented us from detecting any changes in baseline LSNA between groups.

It is also possible that there is an increase in one or more circulating factors such as arginine vasopressin or angiotensin II in OBX rats. Either of these factors could produce a modest elevation in basal MAP and may also be a factor in the attenuated sympathoexcitatory responses after removal of the olfactory bulbs. Recent data indicate that angiotensin II may act centrally to attenuate sympathetic baroreflex responses in a manner similar to the results in the present study (22). Future studies are needed to determine whether neurohumoral factors are responsible for the attenuated sympathoexcitatory responses in OBX rats.

There are several possible reasons why removal of the olfactory bulbs in rats does not result in altered HR responses in the presence of attenuated sympathoexcitation. Because both the parasympathetic and sympathetic nervous systems control HR, it is possible that the two branches of the autonomic nervous system are affected differentially by removal of inputs provided via the olfactory bulbs. Therefore, changes in control of sympathetic nervous system activity specifically cannot be adequately evaluated by recording changes in HR. In addition, HR reflects measurement of an end-organ response to autonomic stimuli, and this response may also be altered.

Interestingly, there is increasing evidence linking mood disorders with cardiovascular disease (8, 20). Little mechanistic data exist to explain this association.
between depression and cardiovascular disease, although changes in autonomic function have been suggested as a possibility (8, 9). Indeed, changes in autonomic tone increase the risk of ventricular fibrillation and sudden death (24). To the extent that elevated baseline MAP might suggest elevated sympathetic nervous system activity, olfactory bulbectomy may be a useful model to investigate pathophysiological mechanisms underlying lowered mood and cardiovascular alterations.

The functional mechanisms underlying the role of the olfactory bulbs in autonomic control are unknown. Anatomic data indicate that there is a multisynaptic pathway from the olfactory bulbs to the NTS via the ventral taenia tectae and central nucleus of the amygdala (4). It is possible that removal of the olfactory bulbs elicits a change in neural processing at either the level of the amygdala or NTS. Both of these areas are responsible for integrating a large number of environmentally and internally derived sensory inputs and eliciting appropriate autonomic responses. Excitation of neurons at the level of the NTS, where peripheral baroreceptors terminate, elicits a reduction in SNA (6). If the olfactory bulbs are responsible for providing descending inhibitory influences at the level of the NTS, removal of this input would tend to raise the level of excitability of the NTS neurons and thus elicit reductions in sympathetic outflow.

As an alternative interpretation, it is necessary to consider that the effects reported here are not due to specific anatomic interruption of olfactory-sympathetic pathways but rather due to secondary effects associated with the chronic adaptation to the lesion itself. Changes in body weight and MAP were observed in the OBX group, which may ultimately lead to alterations in sympathoexcitatory responses. Further studies are necessary to examine the role of food intake, water consumption, or metabolism changes on sympathoex-

Fig. 7. Mean absolute (left y axis and 4 left bars) and changes from BL (right y-axis and 2 right bars) in MAP (A), HR (B), and LSNA (C) during exposure to smoke in sham (n = 4) and OBX (n = 4) rats. Although both groups of rats exhibited a marked bradycardic, pressor, and sympathoexcitatory response, the increase in LSNA was significantly attenuated in OBX rats. *P < 0.05.
citatory responses to physiological stimuli in OBX rats. There is a possibility that central factors are altered due to removal of the olfactory bulb tissue. Considering that these lesions were performed 3 wk before data collection, central nervous system changes as a result of plasticity may be responsible for the attenuated sympathoexcitatory activity in OBX rats.

A large number of central neurotransmitter changes have been identified after bilateral olfactory bulbectomy. Principal among these is the observation that reductions in norepinephrine and serotonin content are reversed by chronic antidepressant treatment (16, 26, 27). In addition, studies have indicated a reduction in glutamate and aspartate levels, whereas GABA reductions in norepinephrine and serotonin content have been identified after bilateral olfactory bulbectomy. The rostral ventrolateral medulla (RVLM) is a primary site within the brain stem involved in the control of SNA. The neurons of the RVLM receive tonic excitatory and inhibitory amino acid inputs. Increased GABAergic control of the RVLM has been implicated in the reduction in baroreflex-mediated increases in SNA (19). It is possible that increased inhibitory control and/or decreased excitatory amino acid control of the RVLM may be a mechanism responsible for the generalized reduction in reflex-mediated sympathoexcitatory effects in OBX rats. Additional studies are required to evaluate this possibility.

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