Molecular Neurobiology

ISSN0893-7648/95/11(1-3): 47-53/$5.40

Cocaine

Selective Regional Effects on Central Monoamines

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Abstract

Cocaine HCl (0, 10, or 50 mg/kg) was injected into adult male ICR mice ip. Thirty minutes later, the brains were removed, and nine regions were isolated: olfactory bulbs, olfactory tubercles, prefrontal cortex, septum, striatum, amygdala, hypothalamus, hippocampus, and thalamus. Using high-performance liquid chromatography, concentrations of norepinephrine, dopamine, serotonin, and their major metabolites and the metabolite/neurotransmitter ratios were determined as an indicator of utilization. Serotonergic systems responded most dramatically. 5HIAA/5-HT decreases were seen in all the brain regions, except the septum, hippocampus, and olfactory bulbs. In most instances, the alterations were dose-dependent. The most profound changes were seen in the amygdala, prefrontal cortex, hypothalamus, and thalamus. For noradrenergic systems, significant responses were seen only in the amygdala, prefrontal cortex, and hypothalamus, but then only at the lower dose. The dopaminergic responses were more complex and not always dose-dependent. The DOPAC/DA ratio was decreased only in the amygdala and striatum at the lower dose, and the olfactory tubercles at the higher dose. It was increased in the septum. The HVA/DA ratios were decreased in the amygdala, prefrontal cortex, and hypothalamus, but only at the lower dose (like MHPG/NE). The 3MT/DA ratio was decreased in the thalamus at the lower dose and in the olfactory tubercles at the higher dose, whereas it was increased in the prefrontal cortex at the lower dose. The HVA and DOPAC routes of degradation were both utilized only by the amygdala. Thus, cocaine produced its most comprehensive effects in this nucleus, as well as the greatest absolute percentage changes for all three of the monoamine systems studied.

Index Entries: Cocaine; monoamines; brain regions; amygdala.

Introduction

Cocaine's effects on central monoamine systems differ significantly depending on the brain region and neurotransmitter studied. The present article consists of a review of the selective effects of cocaine that we have found on serotonergic, noradrenergic, and dopaminergic systems in diverse regions of the rodent brain (Hadfield and Nugent, 1983; Hadfield and

Milio, 1987a, 1992, 1993). The findings help isolate the neurochemical systems and brain nuclei, which are most dramatically perturbed by cocaine. These may be in greater measure responsible for cocaine's neurological and behavioral sequelae.

Methods

Adult male ICR mice (5–7 wk old) were used exclusively in these experiments (Dominion Laboratories, Dublin, VA). (Mice were chosen as a species because we had recently demonstrated that cocaine increases fighting behavior in isolated mice [Hadfield et al., 1982; Hadfield, 1982] and because we wished subsequently to correlate the biochemical findings of the present study with that model, but we have also performed cocaine experiments in rats, where we confirmed that this agent produces uptake inhibition of catecholamines in synaptosomes after in vivo administration [Hadfield et al., 1980]). The animals were housed in an approved animal facility under the supervision of a doctor of veterinary medicine. The animals were kept under controlled conditions of temperature and humidity, and they received standard lab chow and water ad lib. Cocaine was administered ip at a dose of 10 or 50 mg/kg to 10 animals each. Ten drug-free control animals received physiologic saline ip. Thirty minutes later, the brains were rapidly removed and frozen in liquid nitrogen (–320°C). Nine regions were carefully isolated and dissected—the olfactory bulb (OB) (10 mg/kg only), olfactory tubercles (OT), prefrontal cortex (PC), septum (SP), striatum (ST), amygdala (AMY), hypothalamus (HT), hippocampus (HC), and thalamus (TH). Unfortunately, the amount of tissue obtained from the nucleus accumbens (NA) was inadequate for analysis. Several tissues were chosen for study in preference to multiple time and dose variables. The tissues were then weighed and homogenized in sodium acetate buffer that contained isoproterenol (IP) as an internal standard. The homogenate was filtered and centrifuged, and the monoamines were extracted in ascorbate oxidase before injection into our high-performance liquid chromatography (HPLC) system. These HPLC techniques were previously developed in our laboratory to permit the simultaneous measurement of serotonin (5-HT), dopamine (DA), norepinephrine (NE), and their major metabolites in brain tissue during a single short run without prior clean-up of samples (Hadfield and Milio, 1987b,c; Hadfield et al., 1985, 1986).

Results

The data were recorded as nanograms of monoamine/g brain tissue for: NE, 3-methoxy-4-hydroxyphenyl-glycol (MHPG), DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homo-vanillic acid (HVA), 3-methoxytyramine (3MT), 5-HT, and 5-hydroxy-indole-3-acetic acid (5HIAA). The actual values have been published elsewhere (Hadfield and Milio, 1992). Metabolite/neurotransmitter ratios were calculated from the monoamine levels as an indicator of transmitter utilization. All values were subjected to analysis of variance (ANOVA) and, subsequently, Dunnett *post-hoc* tests to determine individual differences (Table 1). The metabolite/neurotransmitter ratios were converted to percent change of drug values over control values to facilitate evaluation (Figs. 1–3). Occasional values proved to be below the limits of measurement.

Discussion

Serotonergic systems were significantly and profoundly altered in several brain regions (except the septum, the hippocampus, and the olfactory bulbs). The effect was clearly dose-dependent (Table 1 and Fig. 1). Typically, levels of serotonin were elevated, levels of 5HIAA were decreased, and the 5HIAA/5-HT ratios were correspondingly decreased—in

Table 1
Effects of Cocaine on Regional Brain Monoamine Metabolite/Neurotransmitter Ratios

	Dose mg/kg	5HIAA/5-HT	MHPG/NE	DOPAC/DA	HVA/DA	3MT/DA
OB	0	0.45 ± 0.042	0.22 ± 0.029	0.14 ± 0.017	0.26 ± 0.054	0.046 ± 0.006
	10	0.36 ± 0.0338	0.20 ± 0.030	0.14 ± 0.022	0.29 ± 0.047	0.081 ± 0.023
	0					
	50					
OT	0		0.33 ± 0.036	0.037 ± 0.007	0.071 ± 0.008	
	10		0.35 ± 0.048	0.032 ± 0.006	0.058 ± 0.005	
	0	0.52 ± 0.017	0.48 ± 0.044	0.078 ± 0.003	0.127 ± 0.026	0.085 ± 0.009
	50	0.37 ± 0.031[c]	0.44 ± 0.050	0.047 ± 0.003[d]	0.081 ± 0.003	0.037 ± 0.004[d]
PC	0	0.27 ± 0.031	0.42 ± 0.022	0.26 ± 0.06	1.3 ± 0.17	0.33 ± 0.072
	10	0.23 ± 0.015	0.26 ± 0.022[d]	0.18 ± 0.027	0.65 ± 0.20[a]	0.67 ± 0.096[a]
	0	0.36 ± 0.028	0.20 ± 0.025			
	50	0.21 ± 0.023[c]	0.17 ± 0.03			
SP	0			35 ± 4.9		
	10			67 ± 8.9[b]		
	0	0.15 ± 0.015				
	50	0.16 ± 0.016				
ST	0	0.6 ± 0.06		0.073 ± 0.002	0.175 ± 0.013	0.078 ± 0.003
	10	0.5 ± 0.04		0.048 ± 0.003[d]	0.161 ± 0.008	0.078 ± 0.003
	0	0.89 ± 0.049		0.031 ± 0.001	0.099 ± 0.011	0.046 ± 0.004
	50	0.69 ± 0.039[c]		0.022 ± 0.001[d]	0.083 ± 0.005	0.040 ± 0.004
AMY	0	0.29 ± 0.012	0.27 ± 0.038	0.11 ± 0.014	1.22 ± 0.304	0.103 ± 0.027
	10	0.24 ± 0.010[c]	0.15 ± 0.019[b]	0.077 ± 0.005[b]	0.46 ± 0.078[a]	0.070 ± 0.013
	0	0.114 ± 0.016	0.29 ± 0.038	0.052 ± 0.009	0.133 ± 0.030	0.079 ± 0.018
	50	0.043 ± 0.009[c]	0.18 ± 0.047	0.039 ± 0.006	0.183 ± 0.044	0.048 ± 0.011
HT	0	0.44 ± 0.028	0.18 ± 0.012	0.44 ± 0.04	1.14 ± 0.060	0.92 ± 0.14
	10	0.30 ± 0.020[c]	0.14 ± 0.010[b]	0.35 ± 0.031	0.90 ± 0.60[b]	0.89 ± 0.093
	0	0.611 ± 0.059	0.120 ± 0.004	0.30 ± 0.08		
	50	0.354 ± 0.045[c]	0.110 ± 0.017	0.30 ± 0.10		
HC	0	0.62 ± 0.067	0.11 ± 0.008		0.23 ± 0.048	
	10	0.43 ± 0.042	0.083 ± 0.009		0.32 ± 0.12	
	0	0.53 ± 0.061				
	50	0.53 ± 0.23				
TH	0	0.54 ± 0.052	0.21 ± 0.021		1.1 ± 0.16	1.1 ± 0.19
	10	0.35 ± 0.023[c]	0.20 ± 0.028		1.2 ± 0.38	0.63 ± 0.060[a]
	0	0.61 ± 0.066	0.41 ± 0.023	0.37 ± 0.061	0.59 ± 0.055	
	50	0.35 ± 0.030[c]	0.39 ± 0.027	0.33 ± 0.076	0.58 ± 0.089	

[a] $p < 0.05$.
[b] $p < 0.02$.
[c] $p < 0.01$.
[d] $p < 0.001$.

many instances by more than 40% of drug-free control values at the higher dose and for the amygdala, >60%. Moreover, the absolute percent change was typically greater than that noted for the noradrenergic and dopaminergic systems (Figs. 2 and 3). Cocaine may cause greater inhibition and disruption of 5-HT neurons than DA and NE neurons, because it has a

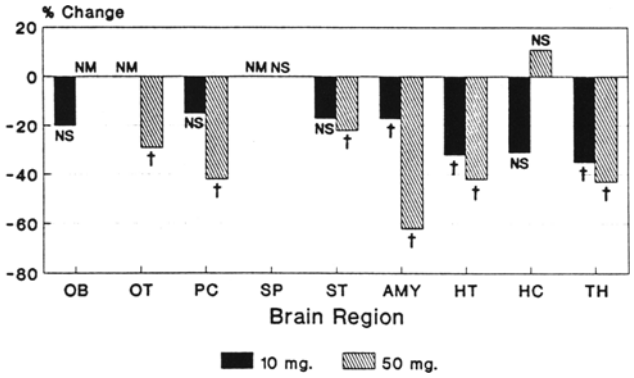


Fig. 1. Percent change in regional 5HIAA/5-HT ratios produced by 10 and 50 mg cocaine HCl (delivered ip 30 min before brain removal). The baseline, drug-free control values (with which the drug values are compared) are set at 0%. All values are derived from Table 1, which includes the standard errors. The actual values for the various monoamine neurotransmitter and metabolite levels obtained in each brain region are published elsewhere (Hadfield and Milio, 1992). Abbreviations used were previously defined in the text except for NM = not measured and NS = not significant. *p* values are: a = <.05; b = <.02; c = <.01; d = <.001.

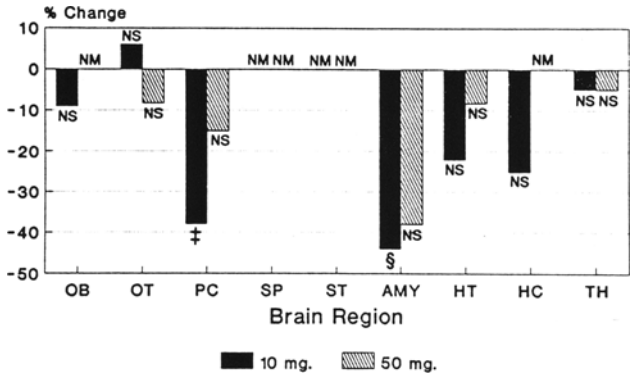


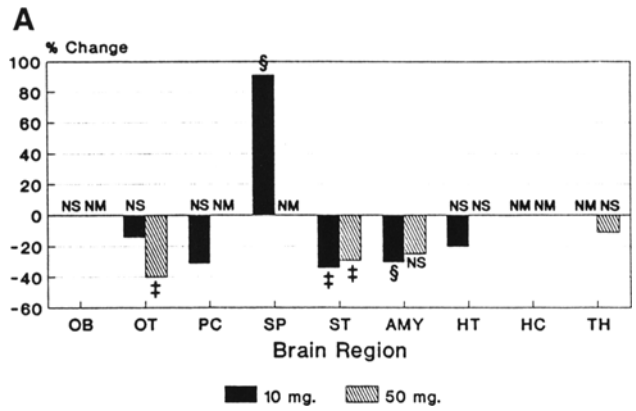
Fig. 2. Percent change in regional MHPG/NE ratios produced by 10 and 50 mg cocaine HCl (delivered ip 30 min before brain removal). The baseline, drug-free control values (with which the drug values are compared) are set at 0%. All values are derived from Table 1, which includes the standard errors. The actual values for the various monoamine neurotransmitter and metabolite levels obtained in each brain region are published elsewhere (Hadfield and Milio, 1992). Abbreviations used were previously defined in the text except for NM = not measured and NS = not significant. *p* values are: a = <.05; b = <.02; c = <.01; d = <.001.

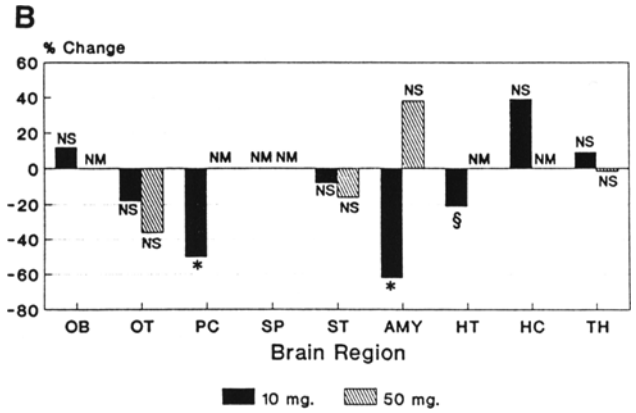
higher affinity for 5-HT transport sites (Gale, 1984; George, 1989).

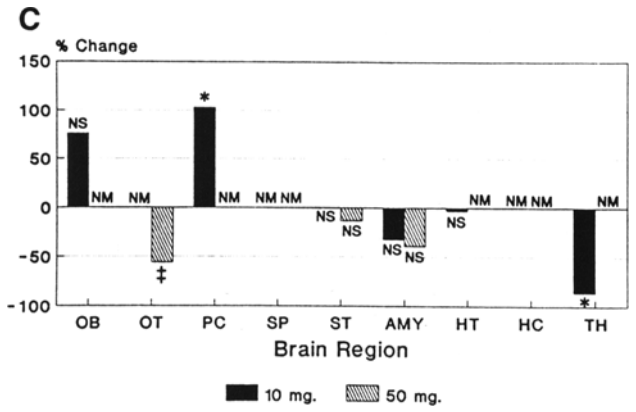
On the other hand, noradrenergic systems were much less affected by cocaine and, paradoxically, the few significant responses noted occurred exclusively at the lower dose (Table 1 and Fig. 2). There was an increased level of NE in the AMY, and a decrease in MHPG and the corresponding MHPG/NE ratios in the AMY, PC, and HT at 10 mg/kg cocaine. Again, the greatest degree of change for the MHPG/NE ratio was in the amygdala, but alterations in the prefrontal (limbic) cortex are also noteworthy. Although no significant changes were seen at the higher 50 mg/kg dose, a trend toward a decrease in the MHPG/NE ratios was noted in these same tissues (AMY, PC, and HT).

This poverty of noradrenergic responses may be because cocaine activates presynaptic α 2-receptors, as reported by Gold et al. (1985). By this mechanism, cocaine would diminish the release of NE as the dose increases. A weak noradrenergic response to cocaine could account for the lack of NE involvement in cocaine reinforcement, as found by deWit and Wise (1977). However, our findings may also be incident to the single postinjection time studied (30 min). Pradhan et al. (1978) showed a biphasic effect of cocaine on NE levels at vari-

Fig. 3. *(opposite page)* Percent change in regional **(A)** DOPAC/DA; **(B)** HVA/DA; **(C)** 3MT/DA ratios produced by 10 and 50 mg cocaine HCl (delivered ip 30 min before brain removal). The baseline, drug-free control values (with which the drug values are compared) are set at 0%. All values are derived from Table 1, which includes the standard errors. The actual values for the various monoamine neurotransmitter and metabolite levels obtained in each brain region are published elsewhere (Hadfield and Milio, 1992). Abbreviations used were previously defined in the text except for NM = not measured and NS = not significant. *p* values are: a = <.05; b = <.02; c = <.01; d = <.001.







ous time intervals. Acute cocaine increased NE levels 10 min following administration, whereas a time interval of 20 min decreased values to normal or below normal levels.

The dopaminergic response to cocaine was intermediate to that of 5-HT, and NE and a clear-cut dose–response effect was not consistently noted. The three major metabolites of DA were studied simultaneously, and the pattern developed in the respective degradative pathways is more complicated (Table 1 and Fig. 3A–C). Levels of the parent neurotransmitter were rarely altered in a significant fashion. DA was increased in the AMY at 10 mg/kg cocaine and in the TH at 50 mg/kg cocaine. On the other hand, the metabolites of DA showed the following changes: (1) DOPAC was decreased in the ST at both the lower and higher doses, and in the OT at the higher dose (Fig. 3A). (2) HVA was decreased in the PC at the lower dose and in the ST at the higher dose, but there was a paradoxical increase of HVA in the TH at the high dose (Fig. 3B). 3MT was decreased only in the ST at the higher dose (Fig. 3C).

When the ratios were calculated, DOPAC/DA values were significantly decreased only in the ST and the AMY at the lower cocaine dose, and paradoxically increased in the SP. The only significant decrease noted at the higher dose was in the OT. HVA/DA values were decreased at the lower dose in the AMY, PC, and HT. However, no differences in HVA/DA ratios were produced by cocaine at the higher dose in any brain region. The 3MT/DA ratios were decreased in the TH, increased in the PC at the lower dose, and decreased in the OT at the higher dose. Note that the AMY was the only tissue in which both the DOPAC and HVA catabolic pathways were exercised.

It is noteworthy that the greatest absolute percent decrease in DA utilization occurred in the ST and AMY. Although the DOPAC/DA ratios were significantly altered in only two other tissues (the OT and SP at a single dose), there was a general downward trend in the absolute DOPAC/DA ratios for all tissues, except the SP. In the SP, cocaine behaved in an anomalous fashion by increasing the DOPAC/DA ratio. Cocaine also failed to affect the serotonergic system in the SP, as reported above. This must be because of a marked difference in receptor populations and/or uptake sites and transporters for this region.

Utilization of DA via the HVA route in the AMY and HT was suppressed by the higher

dose of cocaine. Note that an identical pattern occurred for the MHPG/NE ratios in these same three regions. This may be because of stimulation of DA autoreceptors, which would diminish release of DA and curtail extracellular degradation to HVA. In a related study, we have previously shown that cocaine inhibits DA uptake more significantly in the striatum (A9 DA terminal region) than in the prefrontal cortex (A10 DA terminal region), where there is a paucity of autoreceptors (Hadfield and Nugent, 1983). However, negative feedback processes from the striatum are stronger than those from the prefrontal cortex to the ventral striatum. Alternately, at the high doses used (50 mg/kg), cocaine may have finally suppressed impulse flow activity, an effect that would also lead to the changes. This could be owing to local anesthetic or vasoconstrictor effects of cocaine, which may in turn limit the direct action of this agent on neurons.

From the above, it can be seen that cocaine exerts unique regional effects on the degradation of DA via its various enzymatic routes. Metabolism may be predominantly intracellular or extracellular, depending on the nucleus examined. This signals the presence of important regional differences among DA neurons, as demonstrated in the receptor studies of Goeders and Kuhar (1987).

In summary, we have shown that cocaine produces several important different responses in monoamine systems when they are examined according to discrete brain regions or nuclei, with an emphasis on the amygdala. The role of cocaine on the amygdala is reviewed by Brown et al. (1992). These differential responses to cocaine are most likely the result of local differences in the numbers and/or types of monoamine receptors and transporter molecules found on monoaminergic neurons. The findings are complex and, at present, difficult to interpret in terms of mechanisms underlying cocaine's psychotropic effects, but it is hoped that these data may eventually prove useful in understanding and treating cocaine abuse.

Acknowledgments

The author is grateful for the excellent technical support of Christine Milio. Portions of this material were reprinted from *Pharmacology, Biochemistry and Behavior,* vol. 43, M. G. Hadfield and C. Milio, "Cocaine and Regional Brain Monoamines in Mice," pp. 395–403, Copyright 1992, with kind permission from Pergamon Press Ltd., Headington Hill Hall, Oxford OX3 OBW, UK.

References

Brown E. E., Robertson G. S., and Fibiger H. C. (1992) Evidence for conditional neuronal activation following exposure to a cocaine-paired environment: role of forebrain limbic structures. *J. Neurosci.* **12,** 4112–4121.

deWit H. and Wise R. A. (1977) Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide but not the noradrenergic blocker phentolamine or phenoxybenzamine. *Can. J. Psychol.* **31,** 195–203.

Gale K. (1984) Catecholamine-independent behavioral and neurochemical effects of cocaine in rats, in *Mechanisms of Tolerance and Dependence* (Sharp C. W., ed.), NIDA Res. Mon. **54,** 84–133.

George F. R. (1989) Cocaine produces low-dose locomotor depressant effects in mice. *Psychopharmacology* **99,** 147–150.

Goeders N. E. and Kuhar M. J. (1987) Chronic cocaine administration induces opposite changes in dopamine receptors in the striatum and nucleus accumbens. *Alcohol Drug Res.* **7,** 207–216.

Gold M. S., Washton A. M., and Dackis C. S. (1985) Cocaine abuse: neurochemistry, phenomenology and treatment, in *Cocaine Use in America: Epidemiologic and Clinical Perspectives.* NIDA Research Monograph Series **61,** 1130–1150.

Hadfield M. G., Mott D. E. W., and Ismay J. A. (1980) Cocaine: effect of in vivo administration on synaptosomal uptake of norepinephrine. *Biochem. Pharmacol.* **29,** 1861–1863.

Hadfield M. G. (1982) Cocaine: peak increase in isolation induced fighting post-injection. *J. Neuropharmacol.* **21,** 711–713.

Hadfield M. G., Mott D. E. W., and Nugent E. A. (1982) Cocaine increases isolation induced fighting in mice. *Pharmacol. Biochem. Behav.* **16(2),** 359–360.

Hadfield M. G. and Nugent E. A. (1983) Cocaine: comparative effect on dopamine uptake in extrapyramidal and limbic systems. *Biochem. Pharmacol.* **32,** 744–746.

Hadfield M. G., Crane P., King M. E., Nugent E. A., Milio C., Powell M. D., and Narasimhachari N. (1985) Determination of 13 catecholamines, indolemines, metabolites and precurors in less than 20 minutes during a single HPLC run. *J. Liquid Chromatogr.* **8,** 2689–2697.

Hadfield M. G., Milio C., and Narasimhachari N. (1986) HPLC determination of several monoamines in brain tissue of DBA/2 mice during a single run of 20–25 minutes without prior clean-up of samples. *J. Chromatogr.* **369,** 449–453.

Hadfield M. G. and Milio C. (1987a) Cocaine and regional monoamine utilization in mouse brain. *Soc. for Neurosci.* #464.6 (abstract).

Hadfield M. G. and Milio C. (1987b) Simultaneous HPLC analysis of catecholamines and indoleamines in mouse brain tissue following acetate extraction and treatment with ascorbate oxidase. *J. Liquid Chromatgr.* **10,** 2039–2046.

Hadfield M. G. and Milio C. (1987c) HPLC determination of monoamines in rat brain after enzymatic treatment with ascorbate oxidase and sulfatase. *J. Liquid Chromatgr.* **10,** 2047–2452.

Hadfield M. G. and Milio C. (1992) Cocaine and regional brain monoamines in mice. *Pharmacol. Biochem. Behav.* **43,** 395–403.

Hadfield M. G. and Milio C. (1993) Cocaine and regional brain monoamines. International Society for Neurochemistry: Satellite Symposium: Cellular and Molecular Mechanisms of Drugs of Abuse: Cocaine and Methamphetamine #27 (abstract).

Pradhan S. S., Roy N., and Pradhan S. N. (1978) Correlation of behavioral and neurochemical effects of acute administration of cocaine in rats. *Life Sci.* **22,** 1737–1744.