Effects of Oral Exposure to Mining Waste on in Vivo Dopamine Release from Rat Striatum

Verónica M. Rodríguez, Leticia Dufour, Leticia Carrizales, Fernando Díaz-Barriga, and María E. Jiménez-Capdeville

1Departamento de Bioquímica, Facultad de Medicina and 2Laboratorio de Toxicología Ambiental, Departamento de Biología Celular, Facultad de Medicina, Universidad Autónoma de San Luis Potosí, San Luis Potosí, México

Several single components of mining waste (arsenic, manganese, lead, cadmium) to which humans are exposed at the mining area of Villa de la Paz, Mexico, are known to provoke alterations of striatal dopaminergic parameters. In this study we used an animal model to examine neurochemical changes resulting from exposure to a metal mixture. We used microdialysis to compare in vivo dopamine release from adult rats subchronically exposed to a mining waste by oral route with those from a control group and from a sodium arsenite group (25 mg/kg/day). We found that arsenic and manganese do accumulate in rat brain after 2 weeks of oral exposure. The mining waste group showed significantly decreased basal levels of dihydroxyphenylacetic acid (DOPAC; 66.7 ± 7.35 pg/µl) when compared to a control group (113.7 ± 14.3 pg/µl). Although basal dopamine release rates were comparable among groups, when the system was challenged with a long-standing depolarization through high-potassium perfusion, animals exposed to mining waste were not able to sustain an increased dopamine release in response to depolarization (mining waste group 5.5 ± 0.5 pg/µl versus control group 21.7 ± 5.8 pg/µl). Also, DOPAC and homovanillic acid levels were significantly lower in exposed animals than in controls during stimulation with high potassium. The arsenite group showed a similar tendency to that from the mining waste group. In vivo microdialysis provides relevant data about the effects of a chemical mixture. Our results indicate that this mining waste may represent a health risk for the exposed population. Key words: arsenic, chemical mixtures, dopamine, manganese, metals, mining waste, toxic waste. Environ Health Perspect 106:487-491 (1998). [Online 9 July 1998] http://ehpnet1.niehs.nih.gov/docs/1998/106p487-491/rodriguez/abstract.html

Mining waste accounts for approximately 60% of total industrial waste produced in Mexico (1). Populations living near mining sites are exposed to soil and dust containing metal mixtures as well as to surface water contaminated with mineral debris. In Villa de la Paz, Mexico, mining waste is used as plaster material, and it is therefore sold for finishing houses and buildings.

Several neurotoxic metals are found among mining waste from Villa de la Paz—arsenic, manganese, lead, and cadmium. Although research dealing with the adverse effects of a single exposure to any of these metals is essential to elucidate mechanisms of neurotoxicity, few results from those studies can be extrapolated because exposure is to a mixture, not to the metals alone.

A number of recent studies demonstrate that striatal dopaminergic markers are vulnerable to exposure to several metals. Occupational exposure to manganese results in symptoms resembling Parkinson's disease (2,3). Animal studies have confirmed that striatal dopaminergic neurons are one of the main targets for manganese neurotoxicity (4–6). Arsenic-induced decrements (7) or decrements (8) of striatal dopamine have been reported for rodents. Lead also influences dopaminergic systems, as has been shown by animal studies of D1 and D2 receptors (9,10), dopamine turnover rates (11), behavioral tests (12–14), enzymatic assays (15,16), and in vitro and in vivo release rate studies (17). Similarly, cadmium has been reported to interact with the striatal dopaminergic system (18,19).

In this study, we used an animal model to examine possible neurochemical changes resulting from exposure to mining waste consisting of a mixture of metals. If several single components of the mining waste induce alterations of striatal dopaminergic parameters, it is plausible that a central nervous system alteration provoked by a mixture of metals would be reflected in changes in striatal dopamine. We conducted a microdialysis study of dopamine release in adult rats subchronically exposed to the mining waste by oral route. We chose dopamine release measurements as an index of brain alterations because neurotransmitter release rates are tightly regulated. Therefore, if the synapse is not able to compensate for externally induced modifications to keep transmitter release at the required levels, this is a reliable sign of synaptic alterations.

Materials and Methods

Animals. Sixty male Wistar rats bred in-house weighing 300–350 g were assigned to three experimental groups of 20 animals each. Animals were housed in groups of 6 or 7 during 14 days under controlled conditions of light and temperature with 15 g of food per rat and water ad libitum.

Mining waste administration. Given that arsenic is the metal with the highest concentration in the mining waste (Table 1), it was considered the guide pollutant. Considering the concentration of arsenic and its approximate bioavailability in mining wastes (around 15% (20)), pellets of normal lab diet (Lab Diet, St. Louis, MO) mixed with the mining waste were prepared to administer 0.92 g of the mining waste per day, which corresponds approximately to an intake of 5 mg/kg/day arsenic. One group was exposed for 2 weeks to the mining waste, a second one received an equivalent amount (25 mg/kg/day) of arsenic as sodium arsenite during 2 weeks, and the control group received normal lab chow. As a quality control, the concentrations of arsenic, lead, and manganese in food pellets was verified, with the following results: arsenic 496 ± 184 µg/g, lead 1.04 ± 0.1 µg/g, and manganese 4.44 ± 0.2 µg/g [mean ± standard error (SE)]. Arsenic levels in food pellets prepared with sodium arsenite were not significantly different from those of mining-waste pellets.

Mining waste collection and analysis of metals. A sufficient amount of mining waste was obtained from a mining area in Villa de la Paz, San Luis Potosi, Mexico. The concentration of 10 metals was determined using an inductively coupled plasma (ICP) spectrophotometer (Model 385, Yobin Ion, Lonjumeau, CEDEX France). Separately, arsenic concentration in the mining waste and food pellets was determined using a Perkin-Elmer atomic absorption spectrophotometer (Model 2380, Norwalk, CT) by the hydride evolution–atomic absorption

Address correspondence to M.E. Jiménez-Capdeville, Departamento de Bioquímica, Facultad de Medicina, Av. Venustiano Carranza 2405, 78210 San Luis Potosi S.L.P., Mexico. We acknowledge the technical assistance of J.M. Delgado. This work was supported by grants 0191-N from the Consejo Nacional de Ciencia y Tecnologia (CONACYT) and C96-FAI-07-2.53 from the University of San Luis Potosi. V.M. Rodríguez was supported by a fellowship from CONACYT (92291).

Received 10 October 1997; accepted 6 April 1998.
technique (21–23). Lead and manganese were determined in food pellets and tissue using the graphite furnace absorption technique (24). For all the analyses the mining waste was solubilized with a nitric– perchloric acid mixture.

Surgical and microdialysis procedures. Control and exposed animals were anesthetized with pentobarbital (Anestesal, Pfizer, Mexico, 25 mg/kg, ip), acepromazine (Calminvet, Vetoquinol, Mexico, 0.68 mg/kg, ip), and ketamine (Ketavet, Revetmex, Mexico, 30 mg/kg, ip). Once anesthetized, the animals were placed in a stereotaxic apparatus (Stoelting, Wood Dale, IL). The skull was exposed, and a hole was drilled for placement of a guide cannula over the right striatum (stereotaxic coordinates, anteroposterior: -0.3 mm; lateral: 3 mm; ventral: 4.0, with reference to bregma, according to the atlas of Paxinos and Watson (25)). The cannula was fixed to the skull with anchor screws and acrylic cement. After the surgery, rats were individually housed with free access to water and food during a 48-hour recovery period.

For the microdialysis experiments, a probe of concentric design (CMA/10; Carnegie Medicine AB, Stockholm, Sweden), outer diameter 0.5 mm, and 3-mm dialyzing membrane was inserted into the guide cannula. The dialysis probe was continuously perfused at a flow rate of 2 μl/min through a liquid swivel from a microinfusion pump (74900 series; Cole Parmer, Niles, IL) with a solution containing 147 mM NaCl, 4.0 mM KCl, and 1.2 mM CaCl₂, pH 6.0–6.5. Sample collection was performed every 20 min and started 1 hr after the beginning of the perfusion. After three baseline samples, a solution with high potassium content (91 mM NaCl, 60 mM KCl, 1.2 mM CaCl₂, pH 6.0–6.5) was infused through 1 hr (three samples), and standard Ringer solution was restored for the last four samples. Monoamine content was immediately determined in the dialysates or stored at -20°C until quantification. For verification of the probe’s efficiency, in vivo recoveries were performed before and after each dialysis experiment by placing the probe in a solution containing 100 pg/μl of each of the monoamines and obtaining three consecutive samples of 20 min each (flow rate 2 μl/min). Recovery rates for the monoamines were as follows: dihydroxyphenylacetic acid (DOPAC) 29.8 ± 2.1, homovanillic acid (HVA) 26.1 ± 1.3, and dopamine 22.32 ± 1.2 (mean ± SE; n = 56). Data were not corrected for recovery, and probes were discarded at recovery ratios lower than 20%.

Analysis of dopamine and its metabolites in dialysates. Dopamine and metabolites were quantified in the dialysates by HPLC with electrochemical detection as described in Mejía et al. (7). A Perkin Elmer (series 200) pump was used in conjunction with an electrochemical detector (Bianalitical system LC-4C). A chromatographic column from Alltech Associates Inc. (Deerfield, IL) packed with adsorbosphere (3-mm particle size, 100 × 4.8 mm) was used. The isotropic mobile phase was a phosphate buffer (pH 3.2) containing 0.2 mM sodium octyl sulfate, 0.1 mM EDTA, and 15% v/v methanol (Malinckrodt, Mexico), filtered (0.45 mm pores) and degassed before use. The flow was set at 1.1 ml/min and the determinations were made at room temperature. The electrochemical detector was used at a sensitivity of 1 nA, full scale. The monoamines (DOPAC, dopamine, and HVA) were oxidized with a glassy carbon electrode at a potential of 850 mV relative to the Ag/AgCl reference electrode. The peaks generated by the compounds were analyzed with the software Turbochrom 4 (Perkin Elmer, San Jose, CA). External standards (Sigma, St. Louis, MO) were used to construct a calibration curve for each of the monoamines. Results are expressed in picograms per microliter.

At the end of each experiment rats were sacrificed with an overdose of sodium pentobarbital and were transectionally perfused with 10% buffered formaldehyde. Slices of 10 μm were stained with hematoxylin–eosin to determine the exact location of the dialysis probe. Data from animals with the probe out of the striatum or showing extensive tissue damage were discarded.

Statistics. Data analysis was performed by means of the SPSS/PC program (SPSS Inc., Chicago, IL). After exploratory analysis of normal distribution of the data, we used one-way analysis of variance to detect treatment effects on body weight, brain arsenic concentration, and basal and stimulated release of dopamine, DOPAC, and HVA. Groups of data showing a significant effect of the treatments were further analyzed by means of a multiple comparison procedure (Tukey’s HSD test). Differences among groups were considered significant at p-values <0.05.

Results. Analysis of the mining waste by ICP mass spectrometry showed that its main component is arsenic, followed by manganese and other metals as shown in Table 1. Components of the mining waste readily enter the brain. Figure 1 shows the levels of arsenic and manganese in whole brain of rats exposed to the mining waste during 2 weeks, compared to control and arsenite groups. Arsenic is present in the brain of control animals (38 ± 4.2, mean ± SE), while manganese content builds up after 2 weeks of exposure (147 ± 14.6 ng/g). Lead concentration in the brain was below our detection limit (3 ppb), even after the end of the treatment. The whole-brain arsenic concentration was almost eight times higher in the arsenite group (2,092 ± 186.11 ng/g) than in the mining waste group (269 ± 30.5 ng/g).

Neither the arsenite nor the mining waste group showed significant changes in body weight compared to controls (control group 402.8 ± 8.9 g versus arsenite group 385 ± 19.3 g and mining waste group 383.7 ± 12.4 g: mean ± SE; n = 7–12 animals). During the microdialysis experiments all animals displayed similar behavior. Because experiments were performed during the day, animals were quietly awake, drowsy, or asleep.

Figure 2 shows the temporal courses of dopamine, DOPAC, and HVA release in basal conditions (samples 1–3) under depolarization by perfusion with 60 mM K*. Ringer solution (samples 4–7) and the recovery phase after restoring 4 mM K* (samples

### Table 1. Analysis of mining waste components

| Element | Concentration (ppm) |
|---------|---------------------|
| As      | 9,647               |
| Mn      | 1,650               |
| Zn      | 1,350               |
| Cu      | 1,180               |
| Pb      | 680                 |
| Ni      | 150                 |
| Sb      | 100                 |
| Ba      | 54                  |
| Cr      | 29                  |
| Cd      | 17                  |

*Arsenic content was determined by means of the hydride evol-
tion-atomic absorption technique. The rest of the metals were determined by inductively coupled plasma spectrophotometry.

Figure 1. Content of arsenic and manganese in rat brains. Rats were treated with normal lab diet (control group), sodium arsenite (25 mg/kg of arsenic), and mining waste (0.52 g/day). Each bar represents the mean ± standard error of 6–10 rats.
The mean basal and stimulated release values of dopamine, DOPAC, and HVA for all three groups are shown in Figure 3. Analysis of variance indicated that there was no significant difference in basal dopamine release rates among groups. In contrast, stimulated dopamine release rates showed an effect of the treatment. Control animals displayed a sixfold increase of dopamine release under 60 mM K+ perfusion. Although the arsenite group responded to high potassium with a similar increase in dopamine release, the mining waste group showed only a weak increase in dopamine release in response to a depolarizing stimulus (57% increase).

Concerning DOPAC, basal as well as stimulated levels were significantly lower in the mining waste group as compared to controls (basal: mining waste group 66.7 ± 7.5 pg/μl versus control group 113.7 ± 14.3 pg/μl; stimulated: mining waste group 57.3 ± 6.7 pg/μl versus control group 114.1 ± 15.2 pg/μl). HVA levels, although lower than in control animals, only reached statistical significance for the mining waste controls, although DOPAC levels were significantly decreased. When the system was challenged with a long-standing depolarization through high-potassium perfusion, it became clear that exposure to the mining waste provoked an alteration because exposed animals were not able to sustain an increased dopamine release in response to depolarization. Also, DOPAC and HVA levels were significantly lower in exposed animals than in controls during stimulation with high potassium. Although rats treated with arsenite alone revealed a similar tendency to mining waste-exposed animals, they did not show significant differences in any parameter with respect to controls. This indicates that arsenic alone is not responsible for the effects of the mixture and points to an additive effect of the elements present in the mining waste.

This study sought to establish an animal model for predicting neurological effects by exposure to a chemical mixture. Data from Table 1 indicate that individual analysis of the effects of the mining waste components upon neurotransmitter systems would require a long series of experiments. Furthermore, results from single-exposure testing of a toxic substance can be hardly extrapolated to the real effects of that toxicant in presence of other substances. In this case, arsenic and mining-waste groups were exposed to the same dose of total arsenic; however, brain arsenic content was eight times higher in the arsenite group. This is in agreement with previous estimations of bioavailability of arsenic present in the mining wastes (20,27,28). In contrast, even though the manganese concentration in the mining waste and food pellets was about 100 times lower than that of arsenic, its concentrations in the brain were the same order of magnitude as those of arsenic. Without a previous study of bioavailability of manganese in this mineral mixture, we do not know whether manganese was indeed more bioavailable or if its entrance to the brain was facilitated through other mechanisms, as has been reported for another mixture (7). Similar cases might have been encountered if each metal was analyzed separately; therefore, the direct study of the mining-waste effects appears to be a better alternative for our objective.

In view of the high arsenic content from the mining waste (Table 1), we have recently reported the results of a parallel analysis of arsenic in soil and dust from Villa de la Paz, as well as in urine from children in this population (26). We found 2,904 ± 2,261 ppm in soil and 2,045 ± 1,117 ppm in household dust (mean ± SD). Urinary arsenic concentrations in children were as high as 161 μg/g creatinine, with 69% of the children bearing
more that 100 µg As/g creatinine (26). These findings warrant concern about the neurotoxic potential of the mining waste because we found neurobehavioral alterations in children with arsenic levels of 62 µg/g creatinine (29). Given these facts, it was important to include arsenic as a secondary control in this animal model to obtain data about brain availability of arsenic from the mining waste as well as its possible role in dopamine release.

Data presented in Figures 2 and 3 demonstrate that our microdialysis setup was reliable. First, we obtained basal and stimulated dopamine, DOPAC, and HVA levels as well as recovery rates similar to those reported in the literature (30,31). Second, dopaminergic responses to control preparations were also similar to those reported by others using similar procedures (32–35).

In mining waste-exposed animals, the combined action of metals may compromise the synthesis of dopamine, but efficient modifications of re-uptake and catabolism rates could maintain normal basal release rates. It is plausible that, under basal conditions, a decrease of dopamine synthesis in exposed animals could be counterbalanced by modifications of the re-uptake rates. In this way, less dopamine would be available for metabolism through monoamine oxidase, which would result in decreased basal DOPAC levels (Figs. 2 and 3). Under a situation of higher demand, like prolonged exposure of the striatal cells to high potassium, compensatory mechanisms became insufficient, resulting in significantly lower dopamine, DOPAC, and HVA levels in dialysates.

Another possibility is that exposure to the mining waste is not affecting specific enzymes of dopamine synthesis and metabolism, but that nonspecific membrane alterations caused by the mixture could disturb the normal response to a depolarization through increased release rates, leading to the observed decrease of dopamine, DOPAC, and HVA release under stimulation. Membrane alterations may also modify the activity of membrane-associated enzymes like monoamine oxidase, which is responsible for dopamine conversion to DOPAC, resulting in lower DOPAC basal and stimulated levels. Processes like behavioral activation, motor activity, attention, and learning are all associated with increased release rates of different neurotransmitters in the areas involved in those tasks. For instance, increases of striatal DA release accompanied eating (43%), drinking (22%), and tactile stimulation (15%) (36,37). More important, increases have been observed in acetylcholine (ACh), which is involved in sleep/wake cycles [52% increase during the night (38)]. Also, sensory stimulation has been reported to provoke hippocampal and cortical (ACh) increases ranging from 40% to 220% (39,40). Our observed 600% increases suggest that a 1-hr sustained depolarization with 60 mM K⁺ is not a common physiological situation. Although mining waste-exposed animals showed a marked reduced capability of the dopaminergic terminals around the probe to sustain a prolonged increase of dopamine release, it is still possible that the system can cope with physiological requirements.

In conclusion, we have demonstrated that in vivo microdialysis is suitable for use with an animal model to study neurotoxic effects of a chemical mixture. Our results point to the need for further research that directly characterizes the effects of chemical mixtures to which our population is exposed (41). Also, these results indicate that the mining waste from Villa de la Paz represents a potential health risk for the residents of that area and that measures should be implemented to avoid health problems.

REFERENCES AND NOTES

1. Informe de la Situación General en Materia de Equilibrio y Protección al Ambiente 1991–1992. Mexico, City:Sedesol (Secretaria de Desarrollo Social), 1993.

2. Montgomery EB. Heavy metals and the etiology of Parkinson's disease and other movement disorders. Toxicology 97:3–9 (1995).

3. Iregren A. Using psychological tests for the early detection of neurotoxic effects of low level man- ganese exposure. Neurotoxicology 15:671–677 (1994).

4. Delfazio G, Soleo L, Zafferino R, Livrea P. Manganese toxicity in serums disassociated mesencephalic and striatal primary culture. Brain Res Bull 42:257–262 (1996).

5. Ono J, Harada K, Kodaka R, Sakurai K, Taeji H, Takagi Y, Nagai T, Harada T, Niihe A, Okada M. Manganese deposition in the brain during a long term total parenteral nutrition. J Parenter Enter Nutr 19:310–312 (1995).

6. Shoo WN, van der Sluijs-Gelling AJ, Bramsgaard JB. Selective lesions by manganese and extensive damage by iron after injection into rat striatum or hippo-campus. J Neurochem 62:205–216 (1994).

7. Mejía JJ, Diaz-Barriga F, Calderón J, Rios C, Jiménez-Capdeville ME. Effects of lead-arsenic combined exposure on central monoaminergic systems. Neurotoxicol Teratol 19:489–497 (1997).

8. Nagaraja TN, Desiraju T. Regional alterations in the levels of brain biogenic amines, glutamate, GABA and GAD activity due to chronic consumption of inorganic arsenic in developing and adult rats. Bull Environ Contam Toxicol 50:100–107 (1993).

9. Cory-Slechta DA, Pokora MJ, Fox RA, O’Mara DJ. Lead-induced changes in dopamine D1 sensitivity: modulation by drug discrimination training. Neurotoxicology 17:445–457 (1996).

10. Cory-Slechta DA, relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic, and glutamatergic neurotransmitter systems function. Annu Rev Pharmacol Toxicol 36:301–400 (1996).

11. Lasley SM, Greenland RD, Minnema DJ, Michaelson IA. Influence of chronic inorganic lead exposure on regional dopamine and 5-hydroxytryptamine turnover in rat brain. Neurotoxicol Teratol 16:269–275 (1994).

12. Cory-Slechta DA, Pokora MJ, Preston RA. The effects of lead exposure on hippocampal and cortical (ACh) increases ranging from 40% to 220% (39,40). Our observed 600% increases suggest that a 1-hr sustained depolarization with 60 mM K⁺ is not a common physiological situation. Although mining waste-exposed animals showed a marked reduced capability of the dopaminergic terminals around the probe to sustain a prolonged increase of dopamine release, it is still possible that the system can cope with physiological requirements.

In conclusion, we have demonstrated that in vivo microdialysis is suitable for use with an animal model to study neurotoxic effects of a chemical mixture. Our results point to the need for further research that directly characterizes the effects of chemical mixtures to which our population is exposed (41). Also, these results indicate that the mining waste from Villa de la Paz represents a potential health risk for the residents of that area and that measures should be implemented to avoid health problems.

REFERENCES AND NOTES

1. Informe de la Situación General en Materia de Equilibrio y Protección al Ambiente 1991–1992. Mexico, City:Sedesol (Secretaria de Desarrollo Social), 1993.

2. Montgomery EB. Heavy metals and the etiology of Parkinson's disease and other movement disorders. Toxicology 97:3–9 (1995).

3. Iregren A. Using psychological tests for the early detection of neurotoxic effects of low level man-ganese exposure. Neurotoxicology 15:671–677 (1994).

4. Delfazio G, Soleo L, Zafferino R, Livrea P. Manganese toxicity in serums disassociated mesencephalic and striatal primary culture. Brain Res Bull 42:257–262 (1996).

5. Ono J, Harada K, Kodaka R, Sakurai K, Taeji H, Takagi Y, Nagai T, Harada T, Niihe A, Okada M. Manganese deposition in the brain during a long term total parenteral nutrition. J Parenter Enter Nutr 19:310–312 (1995).

6. Shoo WN, van der Sluijs-Gelling AJ, Bramsgaard JB. Selective lesions by manganese and extensive damage by iron after injection into rat striatum or hippocampus. J Neurochem 62:205–216 (1994).

7. Mejía JJ, Diaz-Barriga F, Calderón J, Rios C, Jiménez-Capdeville ME. Effects of lead-arsenic combined exposure on central monoaminergic systems. Neurotoxicol Teratol 19:489–497 (1997).

8. Nagaraja TN, Desiraju T. Regional alterations in the levels of brain biogenic amines, glutamate, GABA and GAD activity due to chronic consumption of inorganic arsenic in developing and adult rats. Bull Environ Contam Toxicol 50:100–107 (1993).

9. Cory-Slechta DA, Pokora MJ, Fox RA, O’Mara DJ. Lead-induced changes in dopamine D1 sensitivity: modulation by drug discrimination training. Neurotoxicology 17:445–457 (1996).

10. Cory-Slechta DA, relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic, and glutamatergic neurotransmitter systems function. Annu Rev Pharmacol Toxicol 36:301–400 (1996).

11. Lasley SM, Greenland RD, Minnema DJ, Michaelson IA. Influence of chronic inorganic lead exposure on regional dopamine and 5-hydroxytryptamine turnover in rat brain. Neurotoxicol Teratol 16:269–275 (1994).
study of the in vivo release of endogenous dopamine and metabolites. J Neurosci 4:966–977 (1984).
33. Westerink BHC, Hofsteede HM, Damsma G, de Vries JB. The significance of extracellular calcium for the release of dopamine, acetylcholine and amino acids in conscious rats, evaluated by brain microdialysis. Naunyn-Schmiedeberg's Arch Pharmacol 337:373–378 (1988).
34. Moghaddam B, Roth RH, Bunney BS. Characterization of dopamine release in the rat medial prefrontal cortex assessed by in vivo microdialysis: comparison to the striatum. Neuroscience 36:669–676 (1990).
35. Westerink BHC, Tuinte MHJ. Chronic use of intracerebral dialysis for the in vivo measurement of 3,4-dihydroxyphenylethylamine and its metabolite 3,4-dihydroxyphenylacetic acid. J Neurochem 46:181–185 (1986).
36. Mark GP, Rada P, Pothos E, Hoebel BG. Effects of feeding and drinking on acetylcholine release in the nucleus accumbens: striatum, and hippocampus of freely behaving rats. J Neurochem 58:2265–2274 (1992).
37. Adams FS, Schwarting RKW, Boix F, Huston JP. Lateralized changes in behavior and striatal dopamine release following unilateral tactile stimulation of the perioral region: a microdialysis study. Brain Res 553:318–322 (1991).
38. Jiménez-Capdeville ME, Dykes RW. Daily changes in the release of acetylcholine from rat primary somatosensory cortex. Brain Res 625:152–158 (1993).
39. Inglis FM, Fibiger HC. Increases in hippocampal and frontal cortical acetylcholine release associated with presentation of sensory stimuli. Neuroscience 66:81–86 (1995).
40. Inglis FM, Day JC, Fibiger HC. Enhanced acetylcholine release in hippocampus and cortex during the anticipation and consumption of a palatable meal. Neuroscience 62:1049–1056 (1994).
41. Yang RSH, Hong HL, Boorman GA. Toxicology of chemical mixtures: experimental approaches, underlying concepts, and some results. Toxicol Lett 49:183–197 (1989).