Effects of Prenatal Cocaine Exposure on the Developing Hippocampus: Intrinsic and Synaptic Physiology

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Baraban, Scott and Philip A. Schwartzkroin. Effects of prenatal cocaine exposure on the developing hippocampus: intrinsic and synaptic physiology. J. Neurophysiol. 77: 126–136, 1977. A variety of neurological complications has been reported in infants exposed to cocaine during gestation. In the present study, intrinsic cell properties of hippocampal neurons from CA1, CA3, and dentate gyrus regions were measured and compared in tissue from neonatal rats exposed to saline or cocaine in utero. Synaptic properties of the CA1 pyramidal cell region were analyzed at postnatal day (P) 20 with the use of extracellular and intracellular recording techniques. In vitro intracellular recordings (n = 223) obtained at P10, P15 and P20 in tissue from cocaine- and saline-exposed animals revealed no differences in standard cell properties such as resting membrane potential, input resistance, time constant, and action potential amplitude or duration. Hippocampal slices from cocaine-exposed animals exhibited a marked reduction of spike frequency adaptation for all three types of principal hippocampal neurons (e.g., CA1, CA3, and granule cells). The amplitudes of afterhyperpolarizations following a spike train were also decreased in CA1 and CA3 cells in tissue from cocaine-exposed animals. Extracellular and intracellular recordings in the CA1 pyramidal cell region at P20 were obtained to assess and compare synaptic function in tissue from cocaine- and saline-exposed animals. In hippocampal slices from cocaine-exposed animals, synaptic responses in the CA1 region were characterized by multiple population spike activity and reduced inhibitory postsynaptic potentials. The reduction in fast inhibitory postsynaptic potential conductance was not associated with a change in reversal potential. These results suggest that gestational cocaine exposure induces significant changes in intrinsic and synaptic electrophysiological properties of hippocampal neurons in the developing animal. The cell and synaptic features are consistent with an increase in hippocampal excitability, which may contribute to the neurobehavioral deficits and epileptogenic predisposition reported in this infant population. As such, in utero drug exposure model may provide a useful system in which to elucidate and study the basic cellular mechanisms underlying neurological complications associated with maternal cocaine abuse.

INTRODUCTION

In the United States, an estimated 5–15% of pregnant mothers in large urban regions deliver infants who have been exposed to cocaine in utero (Chasnoff et al. 1990; Ellis et al. 1993; Eyler et al. 1994; Kliegman et al. 1994). Some of these cocaine-exposed infants suffer from neurological complications including structural abnormalities of the CNS (Bingol et al. 1987; Cheruki et al. 1988; Gomez-Anson and Ramsey 1994), infant seizure activity (Chasnoff et al. 1990; Kramer et al. 1990; Tsay et al. 1996), and impairment of cognitive abilities (Dow-Edwards et al. 1988; Singer et al. 1993). Cocaine has a low molecular weight and a high water solubility, allowing it to cross the placenta and fetal blood-brain barrier (Myers and Earnest 1984). As such, cocaine or its metabolites may have direct teratogenic effects on the fetus at critical times in development. Experimental studies in rodents have successfully reproduced several clinical manifestations of gestational cocaine exposure on brain growth and function. These features include low birth weight (Akbari et al. 1994; Church et al. 1987, 1990), microencephaly (Royall and Smith 1992), and a general disorganization of cortical laminae (Gressens et al. 1992a,b). In addition, animals exposed to cocaine in utero exhibit 1) increased seizure susceptibility (Snyder-Keller and Keller 1995), 2) impaired learning and memory performance (Spear et al. 1989), and 3) deficits in first-order conditioning tasks (Kosofsky et al. 1994).

The mechanism(s) by which cocaine might induce neurodevelopmental deficits is unknown, although several possibilities have been proposed. First, cocaine is a potent vasoconstrictive agent (Kapur et al. 1991; Thadani 1995), and clinical studies have reported cerebral vascular insults in fetuses born to cocaine-abusing mothers. Either by direct vasoconstrictive effects on fetal circulation or by indirectly causing fetal hypoxia through uterine artery vasoconstriction, prenatal cocaine exposure might compromise sensitive aspects of neuron or glial development. Second, cocaine has deleterious effects on oxidative metabolism (DeVane et al. 1990; Kallyanpur and Ryman 1966). Development and function of the brain is thought to be closely linked to its energy metabolism. Both experimental and clinical data revealed a decrease in brain glucose metabolism in cocaine-exposed offspring (Burchfield and Abrams 1993; Dow-Edwards et al. 1990; Tyler et al. 1993), and this decrease could compromise neurodevelopment. Third, direct inhibitory effects of cocaine on cell replication and DNA synthesis have been demonstrated in the developing rat brain (Anderson-Brown et al. 1990). In neuronal-glial cell cocultures, acute cocaine administration induces neurite perturbations and neuronal death without affecting glial cells (Nassogne et al. 1995). These results would indicate that prenatal cocaine exposure interferes with critical processes in the maturation of the developing CNS.

Although there is strong clinical, epidemiological, and experimental evidence demonstrating deleterious effects of cocaine on the developing fetus, little is known regarding the presence (or absence) of specific changes in cellu-
lar physiology in neonates exposed to cocaine in utero. We have, therefore, begun to examine hippocampal tissue (at various stages of neonatal development) to determine whether prenatal cocaine exposure affects intrinsic and/or synaptic neuronal properties. The results presented here provide the first systematic electrophysiological assessment of neural function in animals exposed to cocaine in utero and suggest that prenatal cocaine exposure can alter the firing properties of principal hippocampal neurons in the neonate.

**METHODS**

**Prenatal cocaine treatment**

Pregnant Sprague-Dawley rats received a daily subcutaneous injection of cocaine (60 mg/kg dissolved in 0.3 ml saline) or saline (0.3 ml) from embryonic day 8 through parturition. This concentration of cocaine is below dosages shown to produce acute seizure activity and lethality in rats (Church et al. 1987; Downs and Eddy 1932) and within the range of drug concentration reported for women who abuse cocaine during pregnancy (e.g., Eddy 1932) and within the range of drug concentration reported and saline-exposed animals (between-group comparisons). Age-matched saline- and cocaine-exposed cohorts of littermates were weighed at birth and on postnatal days 3, 7, 10, 12, and 14. From postnatal day 16 (P16), all rats were culled to 10–12 pups (equal numbers of males and females) and surrogate fostered to nontreated dams who had delivered litters within the preceding 48 h.

**Hippocampal slice preparation**

Cocaine- or saline-treated rat pups were decapitated at three postnatal ages: postnatal days 0–11 (P10); postnatal days 14–16 (P15), or postnatal days 19–21 (P20). The top of the skull was exposed and the brain was chilled with ice-cold, oxygenated slicing medium. The brain was rapidly removed, and one hemisphere was blocked and glued to the stage of a Vibrisclicer (Frederick Haer, Brunswick, ME). Slicing was carried out in chilled (3–4°C), oxygenated sucrose-based artificial cerebrospinal fluid consisting of (in mM) 220 sucrose, 3 KCl, 1.25 NaH_{2}PO_{4}, 2 MgSO_{4}, 26 NaHCO_{3}, 2 CaCl_{2}, and 10 dextrose (295–305 mosM, measured with an osmometer). Sucrose-based slicing medium has been shown to increase cell viability in vitro (Aghajanian and Rasmussen 1989; Baraban and Schwartzkroin 1995). The resulting 400-μm-thick slices were immediately transferred to a holding chamber where they remained submerged in oxygenated recording medium (artificial cerebrospinal fluid) consisting of (in mM) 124 NaCl, 3 KCl, 1.25 NaH_{2}PO_{4}, 2 MgSO_{4}, 26 NaHCO_{3}, 2 CaCl_{2}, and 10 dextrose (295–305 mosM). Slices were held at room temperature for ≥60 min before being transferred to the interface recording chamber and perfused with oxygenated recording medium at 33–35°C.

**Intracellular recording**

Intracellular recordings were obtained from CA1 and CA3 pyramidal cells and from dentate granule cells. Sharp intracellular recording electrodes were pulled from borosilicate glass containing a capillary fiber in the lumen (Sutter Instrument, Novato, CA) and filled with 4 M potassium acetate or 2% biocytin dissolved in 2 M potassium acetate. An amplifier with an active bridge circuit (Neurodata Instruments, New York, NY) was used for intracellular recording. To maintain “standard” recording conditions for comparison of neuronal properties between different experimental groups, we defined the following criteria: 1) intracellular recordings were obtained from a maximum of two neurons per hippocampal slice, 2) the time the slice remained in the recording chamber was limited to <70 min, 3) intracellular recording conditions did not differ with respect to electrode resistance (control: 125 ± 2 MΩ, mean ± SE; cocaine: 120 ± 3 MΩ, P > 0.05), 4) intracellular recording conditions did not differ with respect to temperature (control: 33.4 ± 1.0°C; cocaine: 33.5 ± 0.1°C, P > 0.05), and 5) intracellular recording conditions did not differ with respect to cell depth in the slice measured from the slice surface (control: 112 ± 13 μm; cocaine: 99 ± 8 μm, P > 0.05). Intrinsically neuronal properties were determined at a holding potential of approximately −65 mV. Raw data were recorded on videocassette and analyzed (by an experimenter blind to the origin of the tissue) with the use of pCLAMP software (Axon Instruments, Foster City, CA). Data are presented as means ± SE. As was done previously with similar studies of this type (Rempe et al. 1995; Viana et al. 1995), Student’s independent t-test was used to analyze data from cocaine- and saline-exposed animals (between-group comparisons). Age-dependent comparisons were analyzed with analysis of variance. Significance level was taken as P < 0.05, except as indicated.

**Extracellular recording**

Extracellular recording electrodes were pulled from borosilicate glass and filled with 2 M NaCl (3–15 MΩ). A bipolar stimulating electrode (65 μm center to center) was placed on the surface of the slice in the stratum radiatum. Stimuli consisted of 400-μs constant current pulses of 50–900 μA; paired-pulse intervals were varied between 10 and 90 ms. Spontaneous field activity and responses to stimulation were analyzed on-line and stored on videotape for further analysis.

**Histology**

In some experiments, cells were labeled with 2% biocytin (Molecular Probes, Eugene, OR) by passing 10-nA, 500-ms hyperpolarizing pulses every 500 ms for 5–15 min. After cell labeling, the tissue remained in the chamber for 10–30 min; slices were then fixed in 4% paraformaldehyde between pieces of filter paper and processed as described previously (Buckmaster et al. 1993).

**RESULTS**

Pregnant Sprague-Dawley rats did not exhibit seizure-like activity in the ~40-min period after administration of cocaine. Litter size and survival rate were not different between cocaine-injected and saline-injected mothers. The size (body weight) of cocaine-treated pups (Fig. 1) was consistently smaller than that of controls at all ages tested, as reported previously (Akbari et al. 1994; Xavier et al. 1995). Electrophysiological data were obtained from 48 healthy rat pups between P10 and P20.

**Intrinsic cell properties**

In vitro intracellular recordings from 223 principal hippocampal cells (CA1 pyramidal, CA3 pyramidal, and granule cells) from cocaine- and saline-exposed animals revealed no differences in resting membrane potential, action potential (AP) amplitude, AP duration, input resistance, or membrane time constant (Tables 1–3). However, in
FIG. 1. Neonatal body weight is reduced in rat pups exposed to cocaine in utero. Animals were weighed on postnatal day (P) 10, P15, and P20. Black bars: saline-exposed animals. Shaded bars: cocaine-exposed animals. Values are means ± SE from 48 experiments (P10, n = 12; P15, n = 16; P20, n = 20). Significance taken as \( P < 0.05 \) (*).

Comparing neurons from cocaine-treated and control animals at three postnatal ages (P10, P15, and P20) we found significant and consistent differences in other intrinsic physiology properties.

SPIKE FREQUENCY ADAPTATION. All three types of hippocampal neurons from cocaine-treated animals exhibited reduced spike frequency adaptation (SFA). Under normal recording conditions, hippocampal principal neurons respond to injection of a square depolarizing current pulse with rapid AP discharge, followed by a period of relative silence (Fig. 2, A1, B1, and C1). In cocaine-exposed animals, a depolarizing step elicited a train of APs with much less adaptation (Fig. 2, A2, B2, and C2). SFA is a function of the level of injected current and the duration of the current pulse (Madison and Nicoll 1984). A mean SFA time constant for each neuron was calculated during a 500-ms pulse at current amplitudes between 0.5 and 0.6 nA; resting membrane potential and input resistance did not differ between these two cell groups (Tables 1–3). A plot of the natural logarithm of firing frequency versus latency to individual interspike intervals (from current step onset) was made for each neuron, and the data were fit with a least-squares regression line; the negative reciprocal of this regression line slope was taken as the SFA time constant (Buckmaster et al. 1993). SFA time constant plots for representative CA1, CA3, and granule cell neurons at P15 are shown in Fig. 2. Note the decrease in SFA time constant for all cell types in slices after prenatal cocaine exposure (Fig. 2, A3, B3, and C3).

CA1 pyramidal cells from normal (control) tissue displayed an age-dependent decrease in SFA (Fig. 3, A1 and B; Table 1); this developmental change was not seen in CA3 pyramidal or granule cells in slices from control animals (Tables 2 and 3). CA1 and CA3 pyramidal neurons from cocaine-treated animals also showed an age-dependent decrease in SFA (Tables 2 and 3). Despite these changes, within each group of cells (e.g., CA1 pyramidal neurons at

### TABLE 1. Intrinsic neuronal properties of CA1 pyramidal cells

<table>
<thead>
<tr>
<th>N</th>
<th>RMP, mV</th>
<th>( \text{R}_{\text{in}}, \text{M}\Omega )</th>
<th>( \tau_c, \text{ms} )</th>
<th>AP Amp, mV</th>
<th>AP Dur, ms</th>
<th>AHP Amp, mV</th>
<th>AHP Time to Peak, ms</th>
<th>SFA ( \tau_c, \text{ms} )</th>
<th>DAP, mV</th>
<th>Sag, mV</th>
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<tr>
<td>Control</td>
<td>10</td>
<td>-59.3 ± 2.4</td>
<td>80.2 ± 15.5</td>
<td>30.3 ± 1.6</td>
<td>60.6 ± 1.7</td>
<td>3.8 ± 0.3</td>
<td>10.1 ± 1.5</td>
<td>175.7 ± 47.9</td>
<td>45.5 ± 8.3</td>
<td>9.5 ± 3.3</td>
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<td>85.3 ± 10.2</td>
<td>38.3 ± 1.4</td>
<td>59.8 ± 2.9</td>
<td>3.5 ± 0.3</td>
<td>8.9 ± 2.7</td>
<td>203 ± 60.1</td>
<td>91.3 ± 20</td>
<td>10.9 ± 2.8</td>
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<td>18</td>
<td>-63.6 ± 3.3</td>
<td>80.4 ± 10.4</td>
<td>37 ± 0.6</td>
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<td>6.8 ± 1.2</td>
<td>61.5 ± 19.6</td>
<td>58.4 ± 8.1</td>
<td>13.7 ± 2</td>
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<td>2.6 ± 0.2</td>
<td>7.8 ± 1.2</td>
<td>109.8 ± 21</td>
<td>94.2 ± 12.3</td>
<td>7.6 ± 0.6</td>
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<td><strong>P20 neurons</strong></td>
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<td>91.6 ± 9.2</td>
<td>37.2 ± 0.9</td>
<td>66.5 ± 1.4</td>
<td>2.4 ± 0.1</td>
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<td>67.3 ± 14.2</td>
<td>79.4 ± 9.1</td>
<td>10 ± 1.1</td>
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<td>Cocaine</td>
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<td>81 ± 10.6</td>
<td>36.9 ± 1.1</td>
<td>70.7 ± 2.7</td>
<td>2.3 ± 0.1</td>
<td>4.4 ± 0.6</td>
<td>43.6 ± 4.9</td>
<td>148.9 ± 15.3</td>
<td>9.5 ± 1.1</td>
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</tbody>
</table>

Values are means ± SE. RMP, resting membrane potential; \( \text{R}_{\text{in}}, \text{M}\Omega \), input resistance; \( \tau_c \), membrane time constant; AP Amp, action potential amplitude; AP Dur, action potential duration; AHP, afterhyperpolarization; SFA \( \tau_c \), spike frequency adaptation time constant; DAP, depolarizing afterpotential; sag, anomolous rectification; P, postnatal day. * \( P < 0.05 \).

### TABLE 2. Intrinsic neuronal properties of CA3 pyramidal cells

<table>
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<tr>
<th>N</th>
<th>RMP, mV</th>
<th>( \text{R}_{\text{in}}, \text{M}\Omega )</th>
<th>( \tau_c, \text{ms} )</th>
<th>AP Amp, mV</th>
<th>AP Dur, ms</th>
<th>AHP Amp, mV</th>
<th>AHP Time to Peak, ms</th>
<th>SFA ( \tau_c, \text{ms} )</th>
<th>DAP, mV</th>
<th>Sag, mV</th>
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<td><strong>P10 neurons</strong></td>
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<tr>
<td>Control</td>
<td>12</td>
<td>-63.4 ± 3.8</td>
<td>68.2 ± 9.2</td>
<td>40.9 ± 2.2</td>
<td>62.8 ± 3.2</td>
<td>2.6 ± 0.2</td>
<td>16.4 ± 1.3</td>
<td>202.5 ± 15.4</td>
<td>18.8 ± 2.2</td>
<td>8.4 ± 3.7</td>
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<tr>
<td>Cocaine</td>
<td>13</td>
<td>-64.5 ± 9.2</td>
<td>103.1 ± 16.3</td>
<td>40.4 ± 2</td>
<td>62.5 ± 2</td>
<td>3.1 ± 0.3</td>
<td>11.8 ± 1.1</td>
<td>186.8 ± 14.2</td>
<td>30.1 ± 3.4</td>
<td>14.8 ± 4.3</td>
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<td><strong>P15 neurons</strong></td>
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<td>Control</td>
<td>15</td>
<td>-59.4 ± 1.6</td>
<td>83.9 ± 8.3</td>
<td>43.6 ± 1.8</td>
<td>66.3 ± 1.7</td>
<td>2.1 ± 0.1</td>
<td>16.4 ± 1.9</td>
<td>160.8 ± 11.9</td>
<td>219 ± 5.6</td>
<td>9.5 ± 1.5</td>
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<tr>
<td>Cocaine</td>
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<td>90.8 ± 18.2</td>
<td>42.1 ± 1.7</td>
<td>62.1 ± 2.7</td>
<td>2.6 ± 0.3</td>
<td>9.1 ± 2.3</td>
<td>132 ± 24.2</td>
<td>47.9 ± 13.5</td>
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<td><strong>P20 neurons</strong></td>
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<tr>
<td>Control</td>
<td>14</td>
<td>-59.7 ± 2.5</td>
<td>101 ± 11.9</td>
<td>43.2 ± 1.3</td>
<td>65.7 ± 1.5</td>
<td>2.3 ± 0.1</td>
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<td>196.1 ± 14.1</td>
<td>20.7 ± 2.9</td>
<td>6.5 ± 1</td>
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<td>Cocaine</td>
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<td>-68.8 ± 4.7</td>
<td>86.2 ± 11.3</td>
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<td>67.9 ± 3</td>
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<td>9.1 ± 1.1</td>
<td>143.4 ± 24.5</td>
<td>64.2 ± 9.4</td>
<td>9.8 ± 2.1</td>
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Values are means ± SE. For abbreviations see Table 1. * \( P < 0.05 \).
TABLE 3. **Intrinsic neuronal properties of granule cells**

<table>
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<tr>
<th></th>
<th>N</th>
<th>RMP, mV</th>
<th>$R_{gc}$, MΩ</th>
<th>$\tau_\alpha$, ms</th>
<th>AP Amp, mV</th>
<th>AP Dur, ms</th>
<th>AHP Amp, mV</th>
<th>AHP Time to Peak, ms</th>
<th>SFA $\tau_\alpha$, ms</th>
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<tr>
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<td>$-58 \pm 5.1$</td>
<td>$63.5 \pm 20.8$</td>
<td>$32.6 \pm 2.6$</td>
<td>$55.4 \pm 4.4$</td>
<td>$3.1 \pm 0.3$</td>
<td>n.a.</td>
<td>n.a.</td>
<td>$26.4 \pm 8.2$</td>
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<td>Cocaine</td>
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<td>$52.4 \pm 4.3$</td>
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<td>n.a.</td>
<td>n.a.</td>
<td>$92.1 \pm 41.3$</td>
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<tr>
<td>Control</td>
<td>8</td>
<td>$-58.3 \pm 4.3$</td>
<td>$62.1 \pm 9$</td>
<td>$35.3 \pm 0.9$</td>
<td>$58.7 \pm 2.2$</td>
<td>$1.9 \pm 0.1$</td>
<td>$10.4 \pm 3.7$</td>
<td>$150.6 \pm 26.3$</td>
<td>$26 \pm 9.3$</td>
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<td>$-60.6 \pm 4$</td>
<td>$70.2 \pm 18.5$</td>
<td>$37.5 \pm 1$</td>
<td>$54.7 \pm 6.7$</td>
<td>$1.4 \pm 0.4$</td>
<td>$3.3 \pm 1.1$</td>
<td>$65 \pm 30.9$</td>
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<tr>
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<td>$-66.9 \pm 3.7$</td>
<td>$115 \pm 9.7$</td>
<td>$36.7 \pm 0.6$</td>
<td>$62.1 \pm 2.8$</td>
<td>$1.9 \pm 0.1$</td>
<td>$11.3 \pm 3$</td>
<td>$222.3 \pm 22.9$</td>
<td>$15.9 \pm 1.3$</td>
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<tr>
<td>Cocaine</td>
<td>10</td>
<td>$-73.7 \pm 11.7$</td>
<td>$88.8 \pm 9.7$</td>
<td>$36.3 \pm 0.8$</td>
<td>$61 \pm 2.9$</td>
<td>$2.1 \pm 0.2$</td>
<td>$10.2 \pm 1.6$</td>
<td>$136.2 \pm 27.7$</td>
<td>$93.5 \pm 21.1$</td>
</tr>
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</table>

Values are means ± SE. n.a., not applicable; for other abbreviations, see Table 1. DAP and sag were not consistently observed in granule cells at any age tested. In 3 granule cells the SFA time constant plot was fit using 2 exponentials; SFA time constants for these cells were $9.8 \pm 2$ ms and $194.5 \pm 8.7$ ms, respectively (see Fig. 2C3). For group comparisons a single time constant was obtained from the average of these 2 SFA time constants.

* $P < 0.05$.

P20), adaptation was consistently less in neurons from animals exposed to cocaine in utero compared with saline-exposed control (compare Fig. 3, A1 and A2). A second method for quantification of SFA was used on a group of CA1 pyramidal neurons to verify the cocaine-mediated change in firing properties. The number of APs generated during a single 500-ms depolarizing pulse (0.6 nA) was measured at all three postnatal ages; SFA was also shown to be reduced in cells from cocaine-exposed animals in this analysis ($P < 0.05$). Figure 3B quantifies the difference in the number of APs (per 500-ms depolarizing current pulse) measured at P10, P15, and P20 in cells from saline- and cocaine-exposed rats. This decrease in adaptation was observed over a range of current pulse durations from 200 to 700 ms (data not shown). To confirm the identity of these neurons that showed little adaptation (i.e., to ensure that the recordings were not obtained from interneurons), a small number of neurons from each cell group ($n = 15$) was filled with biocytin; all intracellularly labeled neurons were confirmed as either CA1 pyramidal, CA3 pyramidal, or granule cells (example of a CA1 neuron is shown in Fig. 3C).

**SPIKE TRAIN AFTERHYPERPOLARIZATION.** Afterhyperpolarizations (AHPs) following a train of APs were analyzed for CA1, CA3, and granule cells. We measured and compared the peak amplitude of the AHP and the time to peak following a 100-ms current pulse (0.5–0.6 nA) that elicited at least four APs. AHP amplitudes are correlated with input resistance and resting membrane potential (Lancaster and Adams 1986; Storm 1987), and these parameters did not differ between the two cell groups (Tables 2 and 3). Both
FIG. 3. Characterization of SFA in CA1 pyramidal neurons. A: representative traces of CA1 neurons from control tissue (A1) and tissue exposed to cocaine in utero (A2) at 3 different postnatal ages (P10, P15, and P20). SFA decreases with age, even in controls; for any given age, however, SFA was less apparent in the “cocaine” group. B: scatterplot of the number of action potentials (APs) generated during a single 500-ms depolarization (0.6 nA) for all CA1 neurons tested. Filled diamonds: neurons from saline-exposed animals. Open diamonds: neurons from cocaine-exposed animals. Bars: mean values for each group. C: photomicrograph of a biocytin-filled CA1 pyramidal neuron from cocaine-treated tissue (intracellular recording shown in Fig. 2A2).

CA3 pyramidal neurons (at all 3 ages) and CA1 neurons (at P20) from cocaine-exposed animals exhibited significantly smaller peak AHP amplitudes than cells from age-matched controls (Fig. 4, A, B, and D); there was no difference in AHP amplitude for granule cells (Fig. 4C, Table 3). The AHP time to peak latencies were not different between cocaine and control groups (Fig. 4E).

AGE-SPECIFIC CHANGES. Depolarizing afterpotentials (DAPs) were recorded and compared after single APs evoked by a brief current pulse (5 ms, 0.4–0.6 nA). The DAP amplitude, measured as the voltage difference between the DAP peak and baseline, was obtained at a constant time (~10 ms) relative to the AP. At P15, DAP amplitude was reduced in CA1 pyramidal cells from animals exposed to cocaine in utero (Table 1); there were no differences in CA1 DAP amplitudes at P10 and P20, or for CA3 neurons at any age tested (Tables 1–3). Anomalous rectification (‘sag’) was measured as the difference between peak voltage and steady-state voltage (measured at the 400-ms time point of a 500-ms hyperpolarizing current pulse). At P15, CA1 pyramidal cells showed more sag in slices from cocaine-exposed animals than from controls (Table 1); membrane potential sag was not different for CA1 cells at P10 and P20, or for CA3 neurons at any age tested (Tables 1–3).

**Synaptic physiology**

Extracellular and intracellular responses to synaptic stimulation were obtained in the CA1 pyramidal cell region of animals exposed to saline or cocaine in utero. Synaptic responses were compared and measured at P20 because 1) this age is beyond the period of hyperexcitability observed
in normal animals (Moshe 1987, 1993; Schwartzkroin et al. 1995); 2) prominent changes in intrinsic cell properties were observed at this postnatal time (see Figs. 2–4); and 3) stable field potentials can be reliably elicited at this age (Kreisman and Smith 1993).

FIELD POTENTIALS. Synaptic responses in CA1 stratum pyramidale were obtained with extracellular recording electrodes during stratum radiatum stimulation. In tissue from both control and cocaine-exposed animals, the responses typically consisted of a positive-going field excitatory postsynaptic potential (EPSP) with a superimposed population spike (PS) at higher stimulus intensities. In slices from cocaine-exposed (n = 12) and saline-exposed (n = 9) animals, PS amplitude increased with stimulus intensity and a maximum PS amplitude was reached at a stimulus intensity of ~600 μA (e.g., suprathreshold).

Paired-pulse stimulation protocols revealed differences between slices from cocaine- and saline-exposed animals. In paired-pulse stimulation studies (at suprathreshold stimulus intensities) on slices from control animals, the second stimulus usually elicited a single PS that was potentiated relative to the first response at interpulse intervals ≥20 ms (Nathan et al. 1990). In 12 such trials from controls, a potentiated single PS was evoked in 9 cases and 2 PSs were evoked in 3 cases. However, in tissue from cocaine-exposed animals,

the second stimulus of a paired-pulse protocol evoked multiple PSs in 87% of hippocampal slices (1 PS, n = 2/15; 2 PSs, n = 6/15; 3 PSs, n = 5/15; 4 PSs, n = 2/15). Further, the duration of the field EPSP (i.e., the positive-going waveform from which the PS arose) was greater in slices from cocaine-exposed animals than in slices from saline-exposed animals (control: 27 ± 3.1 ms; cocaine: 49.7 ± 1.5 ms; P < 0.001).

Figure 5A depicts representative traces from a paired-pulse stimulation protocol (stimulus intensity ~600 μA; interpulse interval 90 ms) for hippocampal slices from saline-
and cocaine-exposed animals; note the presence of three PSs on the second field EPSP from the "cocaine" tissue (Fig. 5).

Paired-pulse facilitation, measured as the percentage increase in PS amplitude for the second PS compared with the first PS, revealed another difference between slices from cocaine- and saline-exposed animals. Hippocampal slices from both saline-exposed \( n = 7 \) and cocaine-exposed animals \( n = 7 \) exhibited comparable levels of paired-pulse facilitation at interpulse intervals between 20 and 90 ms. However, paired-pulse facilitation was greater in slices from cocaine-exposed animals than in controls at an interpulse interval of 10 ms; either a small amount of facilitation or paired-pulse inhibition was observed in controls at this short interval, but the cocaine slices showed peak facilitation at 10 ms (Fig. 5B).

**Intracellular Responses.** Consistent with the results from our extracellular recordings, intracellular synaptic responses were also characterized by "hyperexcitability" in cells from slices exposed to cocaine in utero. Normal EPSPs and inhibitory postsynaptic potentials (IPSPs) were elicited by Schaffer collateral stimulation in slices from saline-exposed animals, with the amplitude of EPSPs (and the presence of an AP) varying with stimulus intensity. The EPSP was followed by an inhibitory postsynaptic response, presumably composed of a fast γ-aminobutyric acid-A (GABA\(_A\))-receptor-mediated IPSP (f IPSP) and slow GABA\(_B\) receptor-mediated IPSP (Alger and Nicoll 1982; Newberry and Nicoll 1984). The f IPSP conductance was calculated by linear regression analysis of the slope from the current-voltage relationship between the membrane potential deflection at the latency of the peak f IPSP minus the resting conductance (Connors et al. 1988; Luhman et al. 1995; McCormick 1989). Representative current-voltage plots and the corresponding reversal potential plots for CA1 neurons from control and cocaine-treated animals are shown in Fig. 6A and B. The estimated reversal potentials for the f IPSP were not different between slices from saline- and cocaine-exposed animals (control: \(-72.2 \pm 3.4 \text{ mV}, n = 10\); cocaine: \(-76.2 \pm 3.3 \text{ mV}, n = 9; P = 0.41\)). Synaptic responses to low-intensity stimuli to stratum radiatum, subthreshold for AP generation, were also similar in tissue from control and saline-treated animals in terms of the initial aspects of the EPSP. However, the subsequent fast IPSP was smaller in CA1 neurons from cocaine-exposed animals compared with controls. The peak conductance of the fast IPSP, measured at a fixed time point after stimulation \( (\sim 24 \text{ ms}) \) was significantly smaller \( (P < 0.01) \) in neurons from cocaine-exposed animals compared with controls (Fig. 6C); f IPSP conductance measured at the peak of the response \( (10–30 \text{ ms after the stimulus}) \) was also significantly reduced (control: \( 23.8 \pm 5.8 \text{ nS}; \) cocaine: \( 7.5 \pm 2.4 \text{ ms}; P < 0.01) \).

**Discussion.** The deleterious effects of prenatal cocaine exposure on infant development are well established (Chasnoff 1985; Dixon and Bejar 1989; Singer et al. 1993), but it is unclear what (if any) changes in cellular physiology occur in the neonate. Our results provide the first evidence for specific changes in intrinsic and synaptic physiology in animals exposed to cocaine in utero. Specifically, data from neonatal rats indicates that firing properties of principal neurons in the hippocampus are altered after prenatal cocaine exposure. In particular, these cells show a consistent reduction in 1) SFA (CA1 and CA3 pyramidal cells and granule cells), 2) AHPs following a spike train (CA1 and CA3 pyramidal cells), and 3) synaptic inhibition (CA1 pyramidal cells). These changes in hippocampal physiology, which result in general tissue hyperexcitability, are believed to result from drug exposure during a critical stage of neurodevelopment and may underlie a variety of neurobehavioral deficits in the neonate.

**Intrinsic membrane excitability is altered in hippocampal neurons following gestational cocaine exposure.**

Standard intrinsic neuronal properties important for the control of membrane excitability include resting membrane...
potential, input resistance, AP amplitude and duration, and membrane time constant. These properties undergo changes during normal postnatal development (e.g., a decrease in AP duration and increase in input resistance between P10 and P20) (Schwartzkroin 1982). If prenatal cocaine exposure delayed neuronal development, we would expect to observe intrinsic cell properties for P20 neurons (from cocaine-exposed animals) that resemble cell properties for P10 neurons (from saline-exposed animals). In this study, standard electrophysiological measures revealed no differences between cocaine-exposed and control animals at any ages tested (Tables 1–3), suggesting that prenatal drug exposure did not produce a general impairment of cellular development. However, careful analysis of intrinsic firing properties at three different postnatal ages (P10, P15, and P20) and for three different cell types (CA1, CA3, and dentate gyrus) did reveal differences between tissue exposed to either cocaine or saline during gestation.

A reduction in SFA was seen at all postnatal ages and for all three cell types after prenatal cocaine exposure, as was a reduction in the amplitude of AHPs following a spike train (in the absence of a change in the time to peak amplitude) for both CA1 and CA3 pyramidal neurons from cocaine-exposed rat pups. Treatment-associated changes were superimposed on expected age-dependent changes in neuronal properties (Tables 1–3) (Schwartzkroin 1982). These intrinsic properties play a critical role in modulation of neuronal excitability. For example, SFA serves a protective function to maintain the cell in a nonexcited state and AHPs serve to suppress further neuronal discharge by hyperpolarizing the cell and shunting depolarizing inputs. The changes associated with prenatal cocaine exposure were both in the direction of reduced control of spike firing and thus could contribute to increased hippocampal excitability.

The finding that principal hippocampal cell types exhibited reduced SFA and AHP amplitudes would suggest a common mechanism for modulation of repetitive firing is impaired in cocaine-exposed animals. SFA and AHPs are blocked by agents that selectively inhibit calcium-activated potassium current (Benardo and Prince 1982; Gustafsson and Wigstrom 1981; Hotson and Prince 1980; Madison and Nicoll 1982; Sah and Isaacson 1995; Storm 1987) and voltage-sensitive M current (Madison and Nicoll 1984). Neuropeptides that antagonize these currents, e.g., catecholamines and acetylcholine, have been shown to modulate SFA and AHPs in a variety of neurons (Adams and Brown 1982; Haas and Konnerth 1983; Horn and McAfee 1980; Lorenzon and Foehring 1995; Madison and Nicoll 1982; Storm 1990). Because the acute effect of cocaine is to block reuptake of catecholamines, a possible explanation for the effects observed in our experiments is that prenatal drug exposure produces a prolonged change in the postnatal expression of these neurotransmitters. Indeed, the expression of tyrosine hydroxylase (a catecholamine synthesizing enzyme) is increased in neurons exposed to cocaine in utero (Akbari and Azmitia 1992) and blood levels of dihydroxyphenylalanine (norepinephrine precursor) are elevated in cocaine-exposed infants (Mirochnik et al. 1991). Seidler and Slotkin (1992) have also demonstrated marked noradrenergic hyperactivity after prenatal cocaine exposure in rats, and proposed that fetal cocaine exposure could lead to changes in the rate of neuronal firing in the offspring. Our data indicating a reduction in SFA and AHP amplitudes after prenatal cocaine exposure are evidence for a postnatal alteration in neuronal firing properties and may represent 1) a functional compensation by the ion channels that are modulated by catecholaminergic neurotransmitters (e.g., Ca\(^{2+}\)-activated K\(^+\) or voltage-activated K\(^+\) channels) or 2) excessive/prolonged activation of catecholaminergic receptors on these hippocampal neurons.

Evidence for hyperexcitable synaptic responses in hippocampal slices from cocaine-exposed animals

Our results suggest that the efficacy of GABAergic mechanisms is reduced in slices from animals exposed to cocaine in utero. CA1 field responses from cocaine-exposed animals differed with respect to the number of PSs evoked by the second stimulus of a paired-pulse protocol. At most interpulse intervals, paired-pulse facilitation was comparable between slices from cocaine and control animals. However, at a short interpulse interval expected to produce little facilitation (or modest inhibition), a pronounced facilitation was observed in slices from animals exposed to cocaine in utero. One possible explanation for the reduced paired-pulse inhibition (at 10 ms) and the presence of multiple PS activity would be an attenuation of GABAergic function. Indeed, intracellular recordings of CA1 neurons from cocaine-exposed animals revealed a dramatic reduction in fast IPSP conductance. A reduction in GABAergic inhibition of CA1 cells, coupled with enhanced SFA and reduced AHPs, would contribute to hyperexcitable responses observed in this population.

Prenatal cocaine exposure has been shown to disrupt synaptic organization (Gressens et al. 1992a,b), and changes in hippocampal synaptic function might be a long-lasting effect of this drug exposure. Our results demonstrate that the prenatal effects of cocaine exposure contribute to hyperexcitable synaptic physiology as late as 20 days after exposure to cocaine. It is possible that compensatory mechanisms to restore the balance of excitation and inhibition may occur in these animals at some later date. However, similar reductions in IPSP conductance observed in other rat models of hyperexcitability have been shown to persist for the lifetime of the animal (Luhmann et al. 1995; Mangan et al. 1995; Rempe et al. 1995). Direct measurement of synaptic physiology in adult tissue exposed to cocaine in utero will address this possibility.

Altered hippocampal physiology may contribute to neurologic complications and infant seizure activity following prenatal cocaine exposure

In clinical studies, behavioral seizures (Chasnoff 1985; Kramer et al. 1990; Tsay et al. 1996) and abnormal “seizurelike” electroencephalographic activity (Brown et al. 1989; Legido et al. 1992) have been reported in infants exposed to cocaine in utero. Neurobehavioral deficits have also been observed in cocaine-exposed infants: increased tremulousness, excessive startle responses, delay of visual maturation, lethargy, and sleep irregularities (Anday et al. 1989; Chasnoff 1985; Coles et al. 1992; Singer et al. 1993; Tsay et al. 1996). However, no information is available on...
the potential cellular changes associated with these neurologic deficits. In this study we used an animal model that mimics prenatal cocaine exposure in pregnant mothers (see METHODS) to reveal changes in neonatal neurophysiological function. These changes (e.g., reduced SFA, AHP, and synaptic inhibition), which in all cases promote hyperexcitability, could underlie neurologic deficits and contribute to a potentially epileptogenic state in animals exposed to cocaine in utero.

Interestingly, a similar pattern of reduced SFA and AHP amplitudes for hippocampal CA1 and CA3 pyramidal cells has recently been reported for genetically epilepsy-prone rats (Verma-Ahuja and Pencek 1994; Verma-Ahuja et al. 1995). Genetically epilepsy-prone rats are characterized by reduced catecholamine levels (Dailey et al. 1991; Jobe and Laird 1991; Laird et al. 1984) and exhibit hyperexcitability (reflected in their high susceptibility to a variety of seizure-inducing stimuli) and spontaneous seizure activity (Faingold 1988; Franck et al. 1989; Reigel et al. 1986). Further, recent studies in the rat have indicated that prenatal cocaine exposure reduces the threshold for pharmacologically induced seizures (Snyder-Keller and Keller 1995).

Conclusions

Altered intrinsic and synaptic electrophysiological properties were observed in neonatal rats exposed to cocaine in utero. In the case of reduced SFA and attenuated fIPSP conductance, significant effects were observed at P20, a time at which cocaine (or its metabolites) is not present (Ellis et al. 1993; Ostrea et al. 1992). These changes, which persist into the neonatal period, indicate altered neuronal function and may underlie CNS abnormalities and enhanced seizure susceptibility in animals exposed to cocaine in utero. Because the number of infants born to cocaine-using mothers is increasing at an alarming rate, studies based on prenatal cocaine exposure in rats may serve as an important model to understand and initiate potential drug treatment for this at-risk infant population.

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