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Evaluation of Stearate-Graphite Paste Electrodes for Chronic Measurement of Extracellular Dopamine Concentrations in the Mammalian Brain

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BLAHA, C. D. *Evaluation of stearate-graphite paste electrodes for chronic measurement of extracellular dopamine concentrations in the mammalian brain.* PHARMACOL BIOCHEM BEHAV **55**(3) 351–364, 1996.—Chronoamperometric procedures, in combination with pharmacological treatments, were used to verify whether stearate-modified graphite paste recording electrodes (SGEs) could measure basal extracellular dopamine (DA) concentrations in the striatum of awake rats over a 3-week period of implantation. Baseline chronoamperometric signals were unaffected by systemic injections of ascorbate (AA) or the monoamine oxidase inhibitor pargyline, or by intraventricular infusions of the AA degrading enzyme AA-oxidase. In contrast, systemic injections of *d*-amphetamine or nomifensine increased, and γ-butyrolactone decreased, the signal in a reproducible fashion over a similar test period. In addition, 6-hydroxydopamine lesions of the nigrostriatal DA pathway attenuated the ability of *d*-amphetamine to increase, and γ-butyrolactone to decrease, the chronoamperometric signal. In separate studies, reverse microdialysis, performed with dialysis probes implanted directly adjacent to SGEs in the striatum, permitted the assessment of electrode selectivity, sensitivity, response linearity, and detection limits to DA. Perfusion of the probe with normal Ringer solution (5 μl/min) decreased the baseline chronoamperometric signal by 10 nA. Comparable decreases in the baseline signal were observed after systemic injections of γ-butyrolactone or medial forebrain bundle infusions of tetrodotoxin, suggesting these decreases reflected depletion of extracellular DA to levels below the electrode's detection limit. Reverse dialysis with high concentrations of AA, DOPAC, 5-HT, or 5-HIAA, failed to reverse the decrease in the chronoamperometric signal induced by dialysis. In contrast, reverse dialysis with a physiologically relevant range of DA concentrations, in rats pretreated with the DA uptake blocker nomifensine, increased the chronoamperometric signal in a linear fashion with a detection threshold of < 20 nM. Combined, these results indicate that the baseline chronoamperometric signals recorded at +0.20 V in the striatum with SGEs do not reflect changes in extracellular concentrations of AA, DA metabolites, or indoles, but rather represent neuronally mediated nanomolar changes in extracellular DA concentrations, even after extended periods of implantation in brain tissue.

Stearate-graphite electrodes Dopamine Chronoamperometry Microdialysis Striatum

RECENT advances in neurochemistry and neuropharmacology owe a great deal to the development of in vivo microsampling techniques, such as microdialysis and electrochemistry. These techniques utilize biosensors constructed of dialysis fibers and graphite-based materials, respectively. Dialysis probes and electrochemical recording electrodes have been used to measure changes in concentration of various catecholamine neurotransmitters such as dopamine (DA), norepinephrine, and epinepherine and their metabolites prior to, during, and after pharmacological, physiological, or behavioral treatments (1,4). In studying the neurobiology of the mesotelencephalic DA system, in vivo microdialysis has gained wide acceptance due mainly to the reliable chemical extraction procedures and quantitative detection methods used to measure the chemical composition of dialysate samples (3). Given the limited long-term viability (48–72 h) of implanted dialysis probes due to gliosis (3) and the suggetion by Robinson and others that microdialysis is poorly suited for a within-subjects

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experimental design, there is a clear need for a complimentary in vivo procedure for monitoring DA in brain extracellular fluid (ECF) in individual animals over extended periods of time (15,30,52). For reasons outlined below, we believe that an electrochemical electrode based on stearate-modified graphite paste may be especially useful for chronic measurement of DA.

Conventional graphite paste electrodes (CGEs) lack the necessary surface properties to discriminate between DA and the major brain electroactive interferents of DA, namely, ascorbic acid (AA) and the DA metabolite dihydroxyphenylacetic acid (DOPAC), all of which oxidize within a narrow range of approximately +0.1 V to +0.4 V using standard voltammetric or chronoamperometric recording procedures. Interpretation of electrochemical data using CGEs is complicated further by the catalytic enhancing effects of AA or glutathione on the DA signal (6,10,18,57). In an attempt to improve the selectivity of CGEs for in vivo electrochemical detection of DA, we modified the electrode surface by incorporating stearic acid into the graphite paste (7). This strategy was based on the assumption that an anionic surface formed from stearate carboxylate moieties would retard electron transfer from the anions DOPAC and AA and shift their respective oxidation potentials to more positive values. Electrochemical characterization of stearate-modified graphite paste electrodes (SGEs) has confirmed the complete separation of the voltammetric DA oxidation wave from those of AA and DOPAC using freshly prepared SGEs in vitro and also identified an expected contribution to the DA signal by AA electrocatalysis (6,29). Fortunately, the catalytic effects of AA and glutathione are abolished in vitro following exposure of SGEs to homogenates of rat brain tissue (6).

Before any electrochemical electrode can gain acceptance as an in vivo DA biosensor, it must meet at least five criteria (62). It must be shown that a) in vivo voltammetric recordings are identical to those obtained with authentic DA in vitro, and b) the identity of the species measured in vivo be confirmed by independent chemical means and concentration estimates of ECF DA be in reasonable agreement with those provided by other in vivo methods, such as microdialysis. Additional criteria require that c) the electrode be sensitive enough to measure impulse flow-dependent DA efflux, d) that the in vivo electrochemical signal be dependent on the presence of an intact and functional DA neuronal system, and e) that responses measured over the term of implantation in vivo conform with the known pharmacology of the DA neuronal system.

Evidence confirming the selectivity and sensitivity of SGEs to DA in vivo has come mainly from pharmacological studies and electrochemical recordings taken within 2–8 h of SGE implantation in the striatum or nucleus accumbens of anesthetized rats. These acute drug studies were designed to manipulate independently brain ECF concentrations of DA, AA, and DOPAC. For example, we and others have shown that the chronoamperometric baseline signal is not affected by elevation of brain AA concentrations following systemic administration of AA, or by local microinfusion of the AA degrading enzyme AA-oxidase. Pargyline treatment, which markedly decreases ECF DOPAC, has either no effect or causes a small increase in these signals (7,8,39,64). Other acute drug studies have shown that both systemic administration of γ-butyrolactone and medial forebrain bundle injections of tetrodotoxin (TTX) significantly decrease the chronoamperometric baseline signal in the striatum and nucleus accumbens indicating the dependence of these signals on normal impulse flow along mesotelencephalic DA axons (8,63). To date, the best evidence in support of the selectivity of SGEs comes from other acute studies showing that perfusion of a dialysis probe adjacent to an SGE in the striatum decreases the chronoamperometric baseline signal to the same extent as that seen with γ-butyrolactone or TTX treatments, and that the baseline signal can be restored by reverse dialysis with DA but not DOPAC or AA (5). Altogether, a number of pharmacological (e.g., DA receptor agonists, antagonists, synthesis, metabolism, and uptake inhibitors; neural toxins, neuropeptides, and cholinomimetics) and physiological (e.g., ionophores, ion channel blockers, and electrical stimulation) manipulations have evoked changes in the electrochemical signal recorded at SGEs in the anesthetized rat that are entirely consistent with the documented effects of these treatments on DA neurotransmission (5,7–9,11,63).

Insofar as these studies have confirmed that SGEs measure DA efflux selectively in brain tissue over relatively short-term periods of implantation in anesthetized rats, the present study was designed to provide similar proof that SGEs can be used to monitor ECF DA over an extended period of implantation in freely behaving rats. Repetitive chronoamperometry and a battery of pharmacological tests, similar to those used to confirm the selectivity of SGEs in the acute preparation, were employed to determine the dependence of the baseline chronoamperometric signal on an intact and functional nigrostriatal DA system and reproducibility of responses measured from SGEs to dopaminergic drugs over an 18-day implantation period in tissue. Additional studies using side-by-side SGEs and dialysis probes, in combination with the DA uptake blocker nomifensine, permitted the direct in vivo evaluation of the selectivity, response linearity, and detection limit of SGEs to DA.

METHOD

Subjects and Surgical Procedures

One hundred and eleven male Long–Evans hooded rats (Charles River, St. Constant, Quebec), weighing 250–350 g at the time of surgery, were used in the present study. Rats were housed at a constant temperature (21°C, 60% relative humidity) and maintained on a 12 L:12 D cycle (on at 0700 h), with food and water available ad lib in the colony and test chambers. All experiments were conducted during the light cycle.

Rats were anesthetized with a combination of ketamine (100 mg/kg IP) and xylazine (7 mg/kg IP) and stereotaxically implanted unilaterally or bilaterally in the striatum with SGEs (151 tested) or, in separate rats conventional graphite electrodes (CGEs, 4 tested) devoid of stearate [see (6) for electrode details] (coordinates with respect to bregma: +1.2 mm AP, +3.0 mm ML, and −4.0 mm DV from dura) (48). Reference and auxiliary electrodes were implanted together with recording electrodes and attached to a head pedestal connector cemented to the skull surface, as described previously (20).

In experiments in which AA-oxidase and TTX were used, rats were coimplanted, respectively, with a single 26 g guide cannula positioned 0.5 mm above the right lateral ventrical (coordinates with respect to bregma: −0.8 mm AP, +1.4 mm ML, and tip of 31 g injection cannula −3.5 mm DV from dura; $n = 8$ rats) and the right medial forebrain bundle (coordinates with respect to interaural zero: +4.5 mm AP, +1.3 mm ML, and injection cannula tip +1.6 mm DV; $n = 8$ rats) (48).

Rats scheduled to undergo IV administration of saline, nomifensine, or AA ($n = 50$ rats) were chronically implanted with a single Silastic catheter into the right jugular vein, as

previously described, prior to stereotaxic surgery and flushed before and after each experimental test session (0.1 ml sterile saline containing 10 units/ml heparin) (20). Catheters were flushed with 0.2 ml of saline containing heparin and streptokinase (1 unit and 2 mg/ml, respectively), in the event of line blockage.

Two separate groups of rats (n = 4/group) scheduled to receive injections of *d*-amphetamine and γ-butyrolactone were first injected with desmethylimipramine (25 mg/kg IP) and then anesthetized 30 min later with Somnotol (50 mg/kg IP). 6-OHDA (8 μg/4 μl/10 min base wt.) lesions of the unilateral nigrostriatal DA projection were made at the level of the right medial forebrain bundle via a 31 g stainless steel cannula; coordinates with respect to interaural zero: +4.5 mm AP, +1.3 mm ML, and +1.6 mm DV (48). SGEs were implanted in the striatum ipsilateral to the side of the lesion 2 weeks after 6-OHDA treatment as described above. Antibiotics (garamycin, 8 mg IM and ampicillin, 50 mg IM) were administered after surgery and twice daily thereafter if signs of postoperative illness were apparent.

Electrochemical Equipment and Recording Procedures

Immediately following surgery each rat was placed in a Plexiglas chamber surrounded by an earth-grounded Faraday cage similar in construction to that described previously (20,49). A flexible recording cable extended from the head pedestal connector to a Hg-filled commutator mounted at the top of the Faraday cage which, in turn, was connected to the electrometer device (Echempro, GMA Technologies Inc., Vancouver, BC). Liquid connection to implanted IV catheters was achieved with 23 g Tygon tubing extending from a catheter connector fixed to the skull of the rat to a dual-channel liquid swivel (Instech Inc., Plymouth Meeting, PA) mounted directly above the commutator.

Recording electrodes were conditioned electrochemically in the brain by applying voltammetric sweeps (−0.15 V to +0.45 V at a ramp rate of 0.01 V/s) at 10-min intervals to each electrode for 1 h followed by 1 h of chronoamperometric recordings using 1-s duration square-wave potential pulses applied at 1-min intervals to each electrode. These conditioning procedures were conducted on the day of surgery (designated as day 1), and each day thereafter, prior to the first test trial of the electrodes on the fourth day after surgery and on the day just prior to the second and third test trial (postimplantation days 11 and 18, respectively). Rats were returned to the colony after each day of electrode conditioning and after the conclusion of each test trial.

A square-wave potential amplitude of 0.35 V was used for all chronoamperometric measurements. The initial pulse potential value was determined empirically for each implanted SGE from the potential corresponding to the trough of the catecholamine oxidation wave (point of maximum diffusion-limiting current) recorded typically between +0.2 to +0.25 V. The initial pulse potential was set to a value 0.35 V lower than this value (typically −0.15 V). If the final potential occurred at a value greater than +0.275 V, the SGE was considered no longer viable and the electrode was eliminated from the analyses. The incidence of electrode failure was < 5% the total number of SGEs tested in this study.

Drugs

Dopamine-HCl, dihydroxyphenylacetic acid, L-ascorbic acid, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, γ-hydroxybutyric acid lactone (γ-butyrolactone, 1.12 g/ml), pargyline-HCl, 6-hydroxydopamine-HBr, desmethylimipramine-HCl, and ascorbate-oxidase (EC 1.10.3.3) were obtained from Sigma Chemical Co. (St. Louis, MO). Nomifensine maleate, *d*-amphetamine-SO_4, and xylazine were obtained from Research Biochemicals Inc. (Natick, MA), Smith-Klein Beecham (Ottawa, ON), and Chemagro Ltd. (Etobicoke, ON), respectively. Somnotol and ketamine-HCl were acquired from M.T.C. Pharmaceuticals (Cambridge, ON). Heparin (Organon Teknika, Toronto, ON) and streptokinase (Kabivitrum) saline solutions were prepared fresh as needed. All antibiotics (garamycin and ampicillin, Schering Canada, Pointe Claire, Quebec) were purchased as sterile solutions. All other chemicals used in HPLC-ED analysis were of analytical grade quality and purchased from various commercial sources.

Experimental Procedures

Experiment 1: in vivo analysis of SGE selectivity to DA using side-by-side microdialysis. Electrochemical selectivity to DA was assessed in vivo in rats chronically implanted in the right striatum with an SGE positioned directly adjacent and parallel to the lumen (4 mm length × 0.34 mm o.d., 64,000 M.W. cutoff, Filtral 12 hemodialysis fiber, Hospal-Gambro, St. Leonard) of a microdialysis probe, half the distance from the fiber tip as described previously (5,49); coordinates with respect to bregma +1.2 mm AP, +3.0 mm ML, tip of the SGE −4.0 mm DV from dura (48).

Twenty-four to 48 h after implantation of the probe/SGE assembly the dialysis probe was perfused at 5 μl/min with a 1.5 mM Na-phosphate buffer containing 147 mM NaCl, 3 mM KCl, 1 mM MgCl, and 1.3 mM $CaCl_2$ (pH = 7.3, referred to as normal Ringer, unless noted otherwise) following at least 1 h of chronoamperometric baseline. After ~50 min of dialysis, the probe was reverse dialyzed with a combination of AA and DOPAC (10 and 5 mM, respectively; n = 4 rats) or with serial perfusions of 5-HT and 5-HIAA (5 mM each; n = 4 rats). Rats perfused with a combination of AA and DOPAC were treated ~50 min afterward with normal Ringer containing 100 μM DA with chronoamperometric recordings continuing throughout the experiment.

Experiment 2: SGE responsivity to DA using side-by-side microdialysis. The response of SGEs to relative changes in DA ECF concentrations was examined in vivo in separate groups of rats by perfusing side-by-side dialysis probes with a range of DA concentrations (0.5–25 μM) after DA uptake had been maximally inhibited with the DA uptake blocker nomifensine. Nomifensine was administered repeatedly over the course of each experiment beginning with a bolus IV injection (2.5 mg/kg) followed by the same dose at 30 min intervals for the first hour and every hour thereafter. At the time of the maximum nomifensine-induced increase in the chronoamperometric signal (see Fig. 1B), the dialysis probe was perfused with a Ca^{2+}-free Ringer solution at 5 μl/min. One hour after the start of dialysis, DA was added to the Ca^{2+}-free medium in concentrations of 6.25, 12.5, or 25 μM (n = 4 rats/concentration) or in serial steps of 0.5, 1.0, 1.5, 2.0 and 2.5 μM (n = 6 rats). The latter experiment was repeated in a separate group of rats (n = 4) with 5 mM AA added to the medium containing DA. Controls consisted of chronoamperometric recordings from SGEs implanted adjacent to dialysis probes that were not perfused over the course of IV nomifensine treatments (n = 4 rats).

HPLC with coulometric/amperometric detection (11) was used to determine DA dialysis extraction concentrations $[DA]_e$ (21) for each reverse dialyzed DA concentration

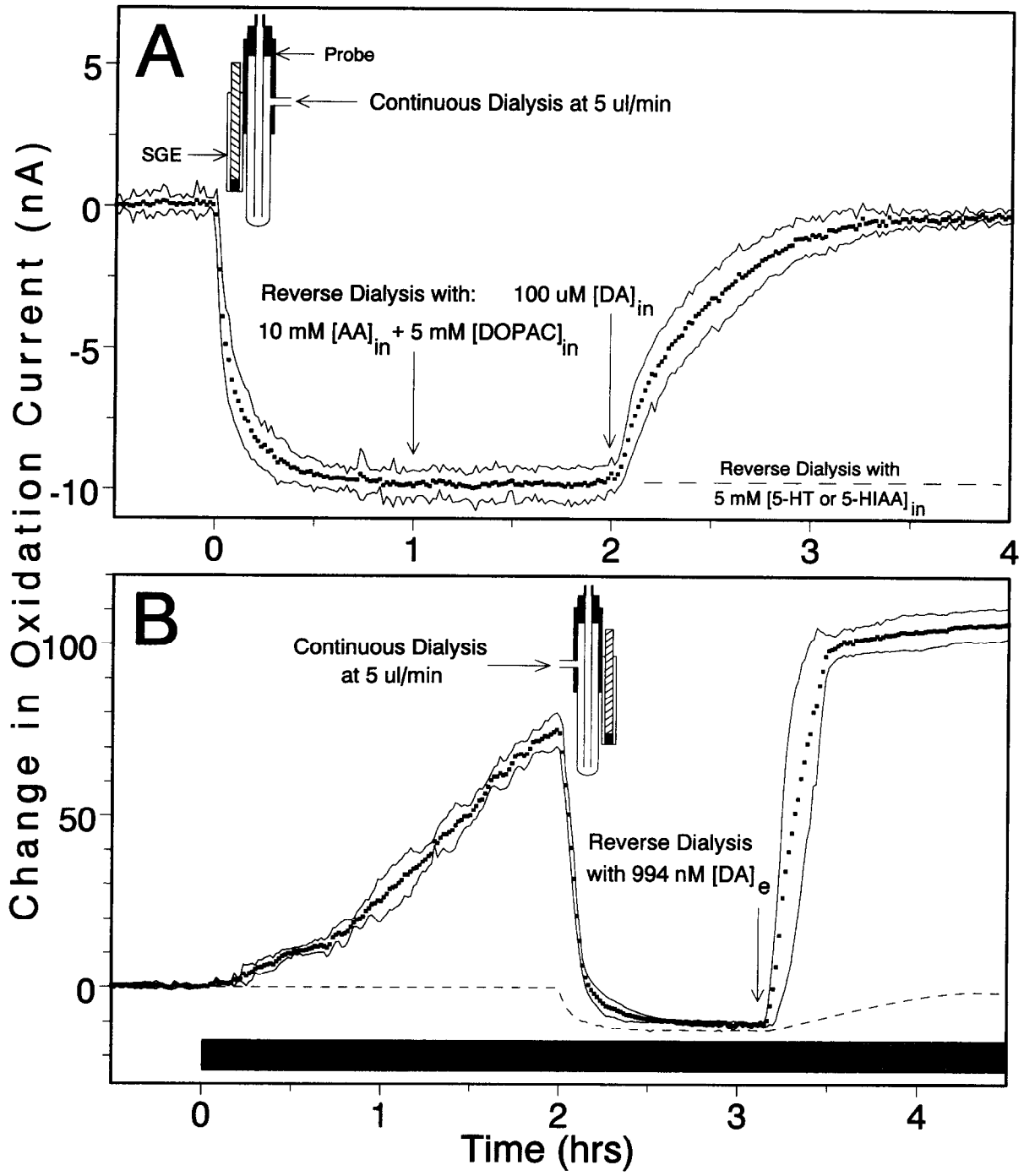

FIG. 1. Chronoamperometric recordings from SGEs positioned adjacent to dialysis probes in the striatum of unanesthetized rats depicting: (A) the reduction of baseline oxidation current (−9.9 ± 0.5 nA) by dialysis with normal Ringer at 5 μl/min and restoration of the signal (−0.3 ± 0.4 nA) following reverse dialysis with 100 μM DA; note that reverse dialysis with AA (10 mM), DOPAC (5 mM), 5-HT (5 mM), or 5-HIAA (5 mM, dashed line) failed to restore the dialysis-attenuated signal; and (B) the nomifensine-induced increase in oxidation current (+71 ± 5 nA), attenuation by dialysis with zero Ca^{2+} Ringer (−10.3 ± 0.6 nA), and reversal of this signal reduction (+106 ± 5 nA) by reverse dialysis with 25 μM DA (estimated mean $[DA]_e$ concentration = 994 ± 35 nM) in the presence of maximal blockade of DA uptake. Dashed line in (B) represents the time course and magnitude of the chronoamperometric response shown in (A). Symbols (■) represent the mean chronoamperometric responses from six rats and solid lines represent the SEM.

thereby providing a measure of DA concentrations at the outer surface of the dialysis probe. $[DA]_e$ and corresponding increases in chronoamperometric oxidation currents were, in turn, used to provide a qualitative estimate of the sensitivity and detection threshold of SGEs to DA. To accomplish this, mean values for DA outflow concentrations $[DA]_{out}$ were determined from three sequential 10-min interval dialysate samples collected 5 min following the steady-state chronoamperometric response to each perfused DA concentration ($[DA]_{in}$). Values of $[DA]_{in}$ were determined from freshly prepared stock solutions of DA injected directly onto the HPLC (n = 3/concentration). Thus, the mean $[DA]_e$ value attained with each $[DA]_{in}$ step was calculated as: $[DA]_e = ([DA]_{in} - [DA]_{out})$.

It is recognized that $[DA]_e$ values represent the concentration of DA at the outer surface of the dialysis membrane only and as such, will be referred throughout the article in these terms. A recent model describing spatial concentration profiles of reverse dialyzed substances suggests that the dialysis extraction concentration decreases exponentially with radial distance from the probe. The degree to which this decrease in concentration occurs in ECF with respect to distance is dependent on the presence of a number of physical (SGE distance from the probe and diffusional resistance, comprised of interstitial volume and tortuosity) and physiological factors (DA metabolism, release, and uptake) affecting DA penetration into the extracellular space (21). In terms of physical factors, it should be noted that the SGE surface was positioned as close as possible to the dialysis membrane surface (estimated distal edge of the SGE surface ~200 μm from the probe surface). Diffusional resistance was not considered to be a significant factor, as the DA that exited the probe was allowed to come into equilibrium with the surrounding tissue before estimating $[DA]_e$ (i.e., the chronoamperometric signal reached a plateau with each dialyzed DA concentration). In terms of physiological factors, DA uptake and release were minimized by combined treatment with nomifensine and reverse dialysis with a calcium-free medium, respectively. By blocking DA uptake, intraneuronal metabolism was also considered to be reduced and extraneuronal metabolism of DA was assumed to play a minor role. However, despite the minimization of these factors, it must be emphasized that DA concentrations at the distal edge of SGE surface will be significantly lower than the value of $[DA]_e$.

Experiment 3: neuronal specificity and impulse flow dependency of SGE oxidation currents. Five separate groups of rats (n = 8/group) were chronically implanted unilaterally with SGEs in the striatum to determine whether baseline chronoamperometric signals were dependent upon the presence and function of DA nerve terminals. The first three groups were drug-naive with one group implanted with a guide cannula aimed at the ipsilateral medial forebrain bundle as described above. The other two groups received 6-OHDA lesions of the right medial forbrain bundle 2 weeks previously as noted above. On the fourth day immediately following 1 h of baseline chronoamperometric measurements, drug-naive rats were administered either *d*-amphetamine (2 mg/kg IP, salt wt.), γ-butyrolactone (750 mg/kg IP), or TTX (10 pmol/1 μl neutral phosphate-buffered saline/1 min into the medial forebrain bundle). 6-OHDA–lesioned rats were given either *d*-amphetamine (2 mg/kg IP, salt wt.) or γ-butyrolactone (750 mg/kg IP). The effect of each drug was monitored over the course of each test day (5–10 h). Rats were sacrificed at the conclusion of these experiments for histological analysis. The right striata of unoperated drug naive (controls, n = 4 rats) and 6-OHDA–lesioned rats were dissected and assayed for DA and DOPAC content (μg/gm wet wt.) by standard HPLC-ED methods to confirm the extent of the lesion.

Experiment 4: pharmacological analysis of SGE response characteristics in vivo. Seven separate groups of rats were implanted bilaterally with SGEs in the striatum to determine long-term selectivity, response variance, and reproducibility to DA, with one of these groups implanted bilaterally with striatal CGEs as noted above. On day 4 after implantation, rats were administered one of the following: group 1, saline (0.3 ml of 1 unit/ml heparinized saline IV, n = 8); group 2, pargyline (75 mg/kg IP, salt wt., n = 4); group 3, AA (100 mg/kg in saline/heparin IV, n = 8); group 4 (SGE implanted), and group 5 (CGE implanted), AA-oxidase (200 units/10 μl neutral phosphate-buffered saline/10 min ICV, n = 6 and 2, respectively); group 6, γ-butyrolactone (200 mg/kg IP, n = 8); and group 7, nomifensine (5 mg/kg saline/heparin IV, salt wt., n = 8). Drug effects were monitored over the course of each test day (5–7 h). Drug treatments with respect to groups were repeated on postimplantation days 11 and 18. Rats were sacrificed at the conclusion of each experiment for histological analysis.

Chronoamperometric Data Presentation and Statistical Analysis

All chronoamperometric time course data are presented as plus or minus mean changes in oxidation current about their respective normalized baseline current values (20). At the conclusion of each test session, the recorded chronoamperometric current value at time zero was adjusted to a current level of zero by subtracting this value from all the other current samples in the electrochemical record. Transformed chronoamperometric data collected for each group of rats receiving a specific treatment on a particular postimplantation day were then averaged and plotted with respect to time. Two-way repeated measures ANOVA was used to determine drug × day interactions over test days 4, 11, and 18. For all analyses, a $p < 0.05$ level was considered significant. All concentration–current curve data were analyzed using linear regression analysis.

Histological Analysis

Upon completion of the experiments, rats were overdosed with ketamine and perfused transcardially with neutral phosphate-buffered saline followed by 10% formalin saline. Brains were removed, stored in buffered 4% neutral formalin for at least 24 h, and then sectioned into 50 μm slices. Every third slice through each brain region of interest was saved and mounted on glass microscope slides. Brains were stained with cresyl violet and electrode and cannulae placements were verified under a light microscope.

RESULTS

Experiment 1: In Vivo Analysis of SGE Selectivity to DA Using Side-by-Side Microdialysis

Perfusion of the dialysis probe with normal Ringer decreased the chronoamperometric baseline signal by ~10 nA within 40 min (Fig. 1A). Reverse dialysis with 100 μM DA provided complete restoration of the signal within 75 min (Fig. 1A) and confirmed that the signal was responsive to changes in DA in the ECF.

Evidence for the selective oxidation of DA at the applied pulse potential of +0.20 V was provided by the complete

inability of reverse dialysis with 10 mM AA and 5 mM DOPAC to restore the attenuated chronoamperometric signal (Fig. 1A). It is worth noting, however, that threefold higher probe concentrations of AA and DOPAC (30 mM and 15 mM, respectively) induced an increase in the signal that plateaued 2 nA above the dialysis-attenuated baseline level (data not shown). In addition, with an applied pulse potential of +0.20V or +0.25V, sequential perfusion of 5 mM of 5-HT and 5-HIAA failed to reverse the dialysis-attenuated signal. The latter pulse amplitude also resulted in a similar 10 nA decrease in the signal during dialysis. In separate experiments however, increasing the pulse amplitude to +0.30 and +0.45 V resulted in dialysis-induced decreases of 14 and 20 nA below baseline, respectively. Moreover, at these two pulse potentials, 5 mM of 5-HT and 5-HIAA each induced elevations in the signal above their dialysis-attenuated baseline levels by 2 and 3 nA, and 16 and 19 nA, respectively (data not shown).

Experiment 2: SGE Responsivity to DA Using Side-by-Side Microdialysis

Consistent with the ability of nomifensine to block DA uptake (48,59), IV administration of nomifensine induced a maximum increase of +68 ± 6 nA in oxidation current with respect to baseline chronoamperometric currents normalized to zero. Repetitive nomifensine injections maintained this level of increase in the signal over the course of the experiment (data not shown). As shown in Fig. 1B, nomifensine induced a similar increase in the signal and at the peak of this increase, initiation of dialysis resulted in a maximal decrease in the signal below the prenomifensine baseline level within 60 min. The magnitude of this decrease with respect to predrug baseline did not differ significantly with that observed in Fig. 1A. However, compared to the effects observed with 100 μM DA (Fig. 1A), reverse-dialysis with 25 μM DA caused a greater reversal in the signal in a much shorter length of time (35 min). In separate experiments, reducing the probe concentrations of DA to 12.5 and 6.25 μM resulted in proportionate increases above the dialysis-attenuated baseline (Fig. 2A and B).

Experiments utilizing lower probe perfusion concentrations of DA showed that, with nomifensine pretreatments and following depletion of the chronoamperometric signal with dialysis, reverse-dialysis with 0.5, 1.0, 1.5, 2.0, and 2.5 μM DA resulted in progressive increases in oxidation current corresponding to cumulative nM increases in $[DA]_e$ (Fig. 2C). It is worth noting, that the reverse-dialysed concentration of 2.0 μM DA, equivalent to an $[DA]_e$ of 86 nM, increased the signal to prenomifensine baseline levels. Moreover, the detection threshold for DA under these conditions corresponded to a $[DA]_e$ of less than 19 ± 6 nM with a signal-to-noise ratio greater than 3:1. These concentration-dependent increases in the chronoamperometric signal resulted in a $[DA]_e$-current curve relationship of 8.5 nM/nA within the linear portion of the curve (Fig. 3A and B).

Replication of the experiment above with 5 mM AA added to the perfusate containing 0.5–2.5 μM DA resulted in a moderate enhancement of the slope of the $[DA]_e$-current calibration curve to a value of ~7 nM/nA (dashed line in Fig. 3B). Whether these augmented effects on the chronoamperometric signal represent an increase due to AA-induced electrocatalysis of DA or a decrease in DA auto-oxidation in the perfusate by AA cannot be specified at this time.

Experiment 3: Neuronal Specificity and Impulse Flow Dependency of SGE Oxidation Currents

Injection of *d*-amphetamine induced an immediate increase in chronoamperometric current reaching maximum values within 60 min of administration (Fig. 4A). This increase was sustained for 2 h with the signal returning to predrug levels within 3.5–4 h. In contrast to these facilitatory effects of *d*-amphetamine, systemic administration of γ-butyrolactone (Fig. 4B) or medial forebrain bundle infusion of TTX (Fig. 4C) resulted in comparable 10 nA decreases in the signal below predrug levels within 2 and 3 h of administration. The γ-butyrolactone–induced decrease in the signal was sustained for an additional 3.5 h, returning to predrug baseline within 8 h of drug injection. In contrast, the decrease in the signal induced by TTX was maintained over the course of the 3 h recording session.

As shown in Fig. 4A and B, unilateral 6-OHDA destruction of DA nerve terminals resulted in a marked attenuation of the excitatory effects of *d*-amphetamine and inhibitory actions of γ-butyrolactone on chronoamperometric currents recorded in the ipsilateral striatum. *d*-Amphetamine–induced increases in lesioned rats were reduced by 80%; however, the overall duration for these effects were comparable to those observed in nonlesioned rats (Fig. 4A). In a similar manner, γ-butyrolactone–induced decreases in the signal were also attenuated by 90% in lesioned rats and exhibited a relatively shorter duration of action of 6.5 h compared to nonlesioned rats. Postmortem analysis of striatal tissue taken from each drug-treated group revealed a > 97% depletion in DA and DOPAC on the lesioned side compared to unoperated drug-naive control rats (Fig. 4 caption).

Experiment 4: Pharmacological Analysis of the SGE Response Characteristics In Vivo

Saline administration had no significant effect on chronamperometric baseline current levels recorded from SGEs over an 18-day postimplantation period in the striatum (Fig. 5, left panels). As well, the variance in mean baseline currents remained relatively small and uniform (SEM range 0.2–1.1 nA) over the 7-h recording period and across test days 4, 11, and 18. The MAO inhibitor pargyline also was without effect on the signal over the 18-day test period (Fig. 5, right panels) and exhibited mean variances about baseline comparable to those observed across test days in the saline-treated group. Moreover, AA also failed to alter significantly baseline signals recorded on days 4, 11, and 18 (Fig. 6, top panels). That these null effects of saline, pargyline, and AA were not due to a loss in SGE sensitivity to DA was confirmed from observations of the effects of *d*-amphetamine on baseline oxidation currents recorded on postimplantation day 19 in the AA-treated group. As shown in Fig. 6 (bottom panel), *d*-amphetamine significantly increased the signal in the striatum within 60 min of administration. Both the amplitude and duration of these increases in the signal were comparable to those observed in *d*-amphetamine-treated rats on postimplantation day 4 (see Fig. 6, bottom panel and Fig. 4A, respectively).

As shown in Fig. 7 (left panels), significant reductions in ECF concentrations of AA via ICV infusions of the enzyme AA-oxidase failed to significantly alter the basal chronoamperometric signal recorded from SGEs in the striatum over the 18-day test period. In contrast, a marked decrease in the signal was observed at CGEs in the striatum following AA-oxidase

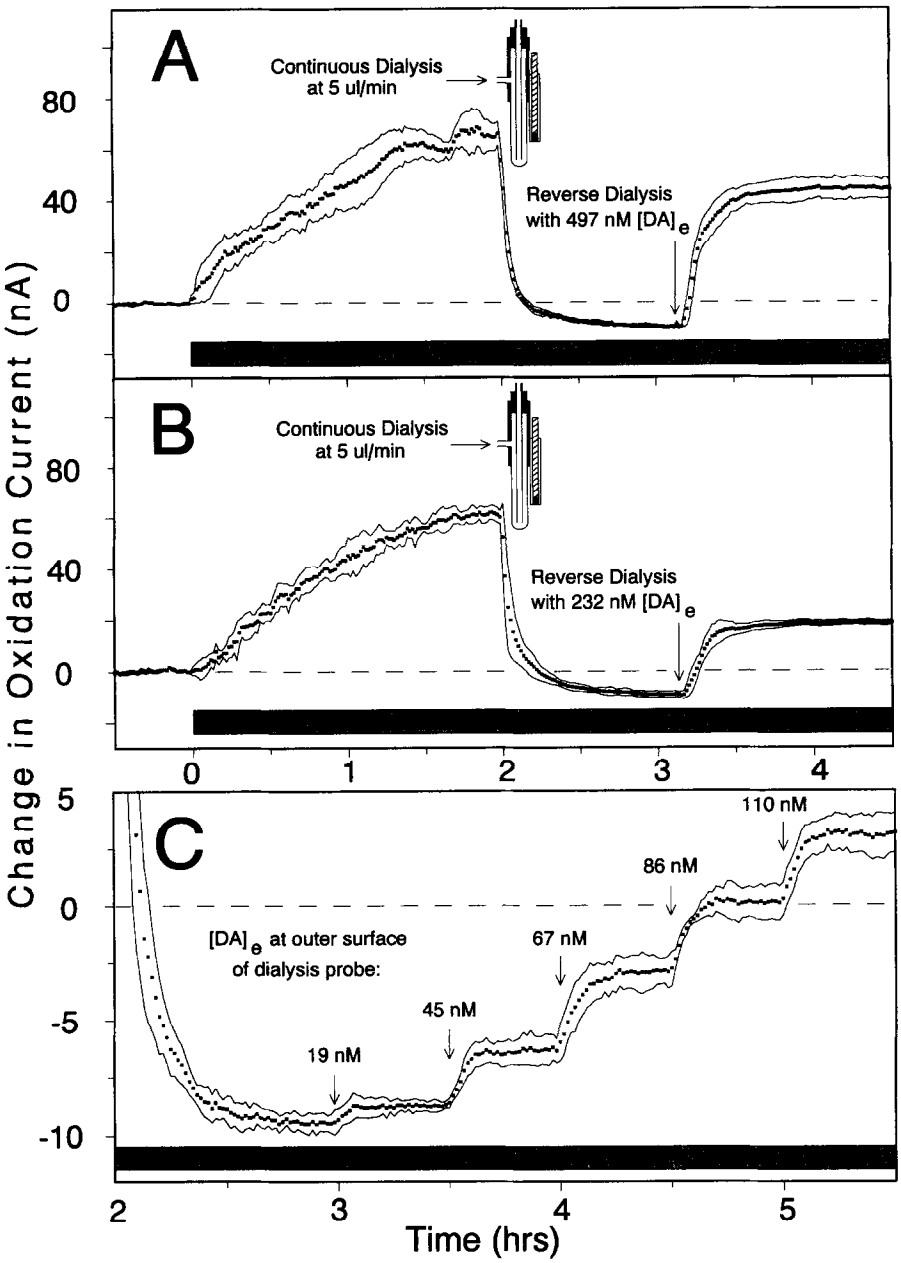

FIG. 2. The effects of microdialysis on maximal mean nomifensine-induced increases in chronoamperometric oxidation current (A, +66 ± 5 nA; B, +62 ± 3 nA; C, +64 ± 7 nA) measured at adjacent SGEs in the striatum of unanesthetized rats, attenuation by dialysis with zero Ca^{2+} Ringer (A, −10.1 ± 0.7 nA; B, −9.6 ± 0.9 nA; C, −9.6 ± 1 nA), and reversal of this signal reduction (A, +44 ± 4 nA; B, +19 ± 1 nA; C, −8.7 ± 0.3, −6.5 ± 0.6, −3.2 ± 0.8, +0.2 ± 0.6, and +3.2 ± 0.7 nA) by reverse dialysis with (A) 12.5 μM, (B) 6.25 μM, and (C) 0.5, 1.0, 1.5, 2.0, and 2.5 μM dialysate DA concentrations. Based on post hoc analysis using HPLC, reverse dialysed $[DA]_e$ correspond to mean values of (A) 497 ± 25 nM, (B) 232 ± 12 nM, and (C) 19 ± 6, 45 ± 3, 67 ± 10, 86 ± 6, and 110 ± 9 nM, respectively. Note that the time at 2 h in (C) corresponds to the time of maximum inhibition of DA uptake by nomifensine. Dashed lines in (A–C) represent prenomifensine baseline. Symbols (■) represent the mean chronoamperometric responses from four (A and B) and six (C) rats, and solid lines represent the SEM.

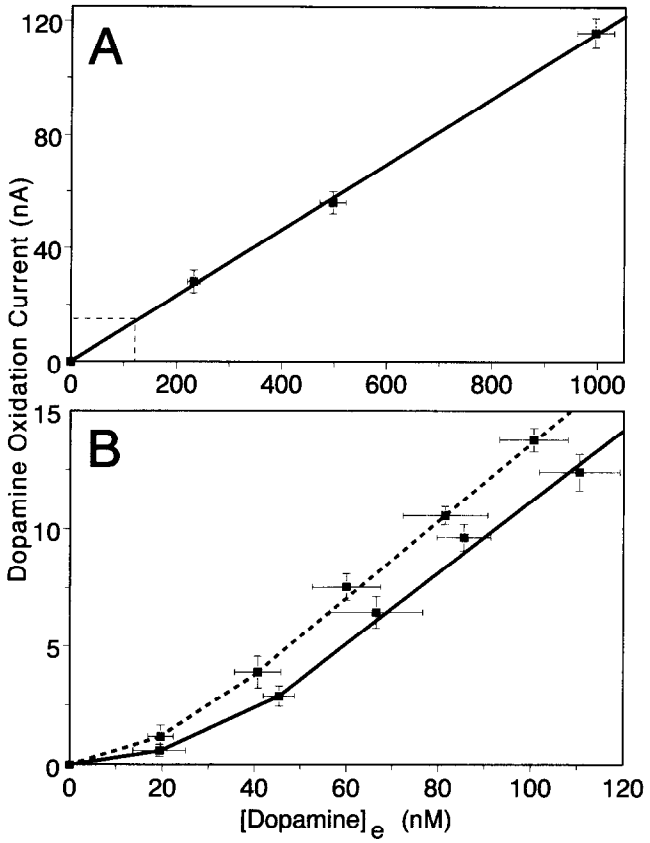

FIG. 3. $[DA]_e$-current calibration curves for DA determined in vivo from the data presented in Figs. 1B and 2A–C. Solid lines represent the mean DA oxidation current (A, $n = 4$; B, $n = 6$) and corresponding $[DA]_e$ achieved by reverse dialysis at the surface of the dialysis probe with $[DA]_{in}$ of (A) 6.25, 12.5, and 25 μM DA and (B) 0.5, 1.0, 1.5, 2.0, and 2.5 μM DA. Vertical and horizontal bars represent the SEM variations for chronoamperometric current increases in relationship to $[DA]_e$, respectively. Note that in (B, dashed line; $n = 4$), inclusion of 5 mM ascorbate within the probe (~200 μM estimated $[AA]_e$) produced a modest enhancement of the responses of SGEs to stepwise additions of dialysate DA concentrations (0.5 to 2.5 μM). Dashed box outline in (A) corresponds to current-concentration scale shown in (B). Slope (A), $r = 0.998$; Slope (B, solid line), $r = 0.945$; Slope (B, dashed line), $r = 0.970$.

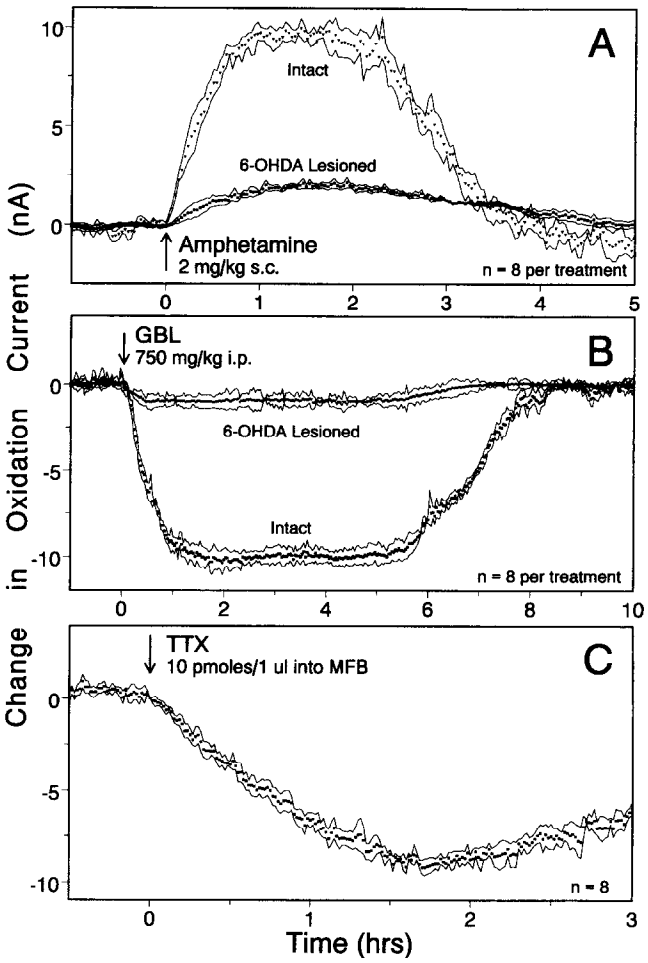

FIG. 4. Time courses of chronoamperometric responses recorded from SGEs in the striatum of freely moving rats following systemic injections of (A) *d*-amphetamine (2 mg/kg SC, +9.96 ± 0.6 nA), (B) γ-butyrolactone (GBL, 750 mg/kg IP, −10.17 ± 0.7 nA), and infusion of (C) tetrodotoxin (TTX, 10 pmol/1 μl/1 min, −9.18 ± 0.72 nA) into the medial forebrain bundle (MFB). Note that unilateral degeneration of DA nerve terminals following 6-hydroxydopamine (6-OHDA) lesions resulted in a significant attenuation of the (A) stimulatatory effects of *d*-amphetamine (+1.98 ± 0.25 nA), $F(1, 14) = 692.84$, $p < 0.001$, and (B) inhibitory actions of γ-butyrolactone (−0.9 ± 0.57 nA), $F(1, 14) = 670.9$, $p < 0.001$, on oxidation currents recorded in the striatum. Symbols (■) and solid lines represent the mean chronoamperometric responses and SEM from eight rats per treatment, respectively. For clarity, every second measurement taken is shown. HPLC postmortem tissue analysis of DA and DOPAC in controls vs. lesioned *d*-amphetamine and γ-butyrolactone groups: DA, 12.63 ± 0.69 vs. 0.32 ± 0.16 and 0.28 ± 0.26 μg/gm; DOPAC, 2.35 ± 0.21 vs. 0.05 ± 0.12, and 0.08 ± 0.09 μg/g, respectively.

injections over a similar test period (Fig. 7, right panels). As CGEs are sensitive to AA (6), and DA or DOPAC levels are unaffected by AA-oxidase (8,12), these data confirm that these AA-oxidase treatments reduced ECF concentrations of AA significantly in the striatum.

Evidence supporting the pharmacological reproducibility of the chronoamperometric response of chronically implanted SGEs was obtained in the final set of experiments. As shown in Fig. 8, both γ-butyrolactone (left panel) and nomifensine (right panel) produced consistently reproducible effects on the baseline signals recorded on days 4, 11, and 18. Maximum decreases in the signal by γ-butyrolactone were attained after 60 min of injection with a recovery to baseline within 4 h. In contrast, nomifensine increased the signal significantly, reaching peak values in 2–2.5 h followed by a gradual recovery to predrug injection baselines by 6–6.5 h.

DISCUSSION

The present experiments provide evidence in support of SGEs as valid biosensors for monitoring DA neurotransmission in the striatum of awake, behaving rats. Overall, the results demonstrate that SGEs meet all of the five recognized criteria used to identify the electroactive compound detected in vivo (62). The first of these criteria requires that the voltammogram monitored in vivo be identical to that of authentic DA recorded in vitro. Although not presented here, the ob-

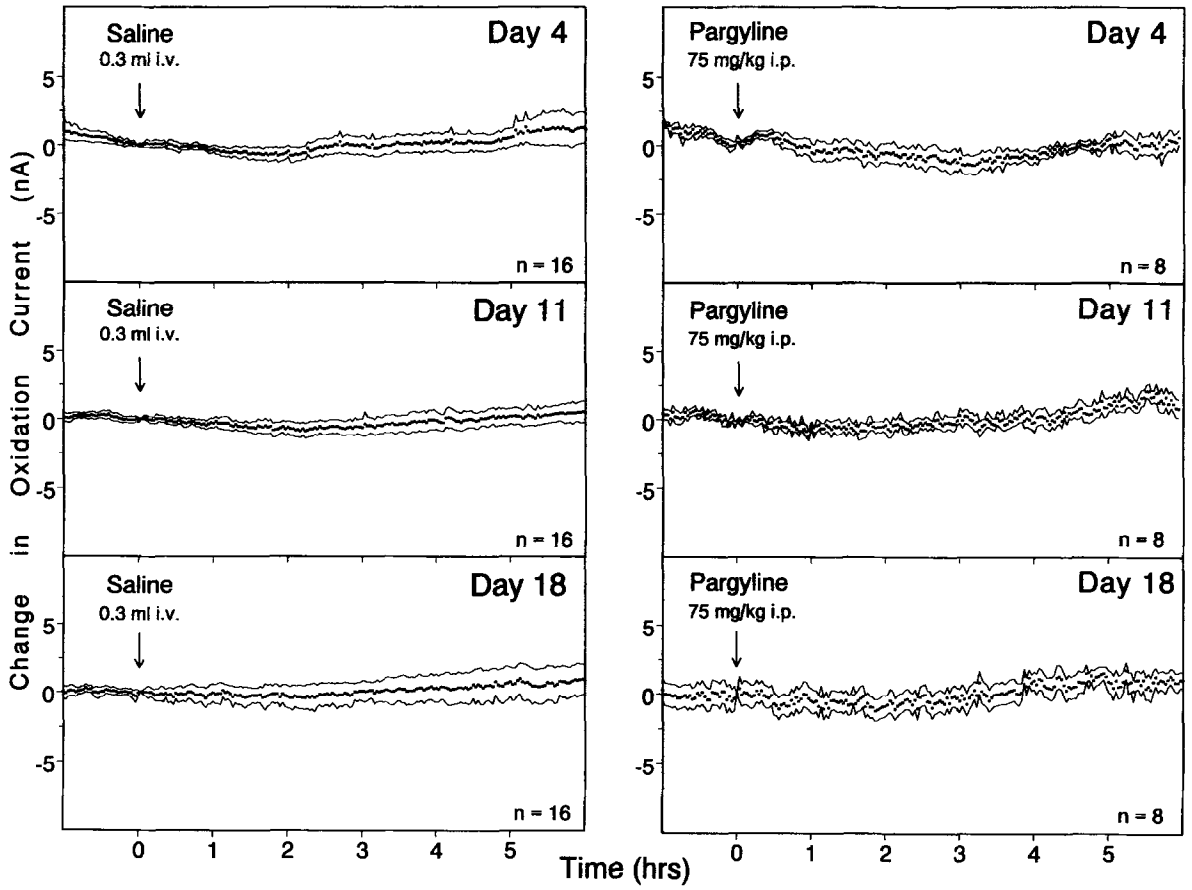

FIG. 5. Mean change in oxidation currents recorded in the striatum of freely moving rats before and after administration of (left panels) saline vehicle (0.3 ml of 0.9% NaCl containing 1 unit/ml heparin IV) and (right panels) pargyline (75 mg/kg IP). Chronoamperometric recordings were conducted on days 4, 11, and 18 following chronic bilateral implantation of SGEs. Symbols (■) and solid lines represent the mean chronoamperometric responses and SEM from 8–16 electrodes per treatment, respectively. Chronoamperometric responses did not differ significantly across all test days for saline, $F(2, 45) = 0.64$, $p = 0.53$, or pargyline, $F(2, 21) = 0.43$, $p = 0.66$. For clarity, every second measurement taken is shown.

served voltammograms corresponding to DA oxidation in the striatum were identical to in vivo voltammograms reported previously (42) under similar recording conditions and to voltammograms of authentic DA recorded in vitro at brain-treated SGEs (6,10). As with most surface-modified carbon-based electrodes, including electrochemically pretreated and/or Nafion-coated carbon fibers (2,24,31,32,58), the catecholamines, norepinephrine and epinephrine, are the only other electroactive compounds that exhibit voltammetry similar to DA with SGEs in brain at these applied potentials. However, the contribution of these compounds to the signal was considered insignificant since their tissue content in the striatum is negligible (60).

A second criterion requires identification of the detected species by independent chemical means. Wightman and colleagues provided independent chemical verification by using mass balance calculations to show that stimulation-induced DOPAC formation in postmortem tissue samples was proportional in magnitude to electrochemically detected ECF DA efflux (62). The present use of the side-by-side reverse microdialysis procedure to alter ECF concentrations of DA and a number of potentially interfering electroactive compounds provided a more direct in vivo method of confirming SGE selectivity to DA. By controlling the concentration and chemical species delivered into the ECF adjacent to SGEs, the chronoamperometric detection of DA was shown to be resolved completely from DOPAC and AA. Of equal importance were the findings of a complete lack of interference from 5-HT or its metabolite 5-HIAA using chronoamperometric pulse amplitudes (+0.2 to +0.25 V) typically employed to detect DA in vivo.

Side-by-side reverse microdialysis also permitted relative changes in DA concentrations and detection thresholds to be examined in vivo. As noted in the Method section above, the qualitative evaluation of these parameters required concomitant blockade of high affinity DA uptake with nomifensine treatments (13). This approach is in keeping with recent electrochemical studies showing the striatum as a brain region that is "uptake dominated" (28), with a significant fraction of DA uptake sites located outside of the synapse (27). These uptake sites may serve as a tissue sink to reduce ECF concentrations of reverse dialyzed DA (21,27,59). This appears to be the case, because the chronoamperometric signal increased at significantly faster rates and achieved greater response magnitudes to much lower reverse dialyzed DA concentrations in the presence of DA uptake blockade (e.g., Fig. 1A vs. B).

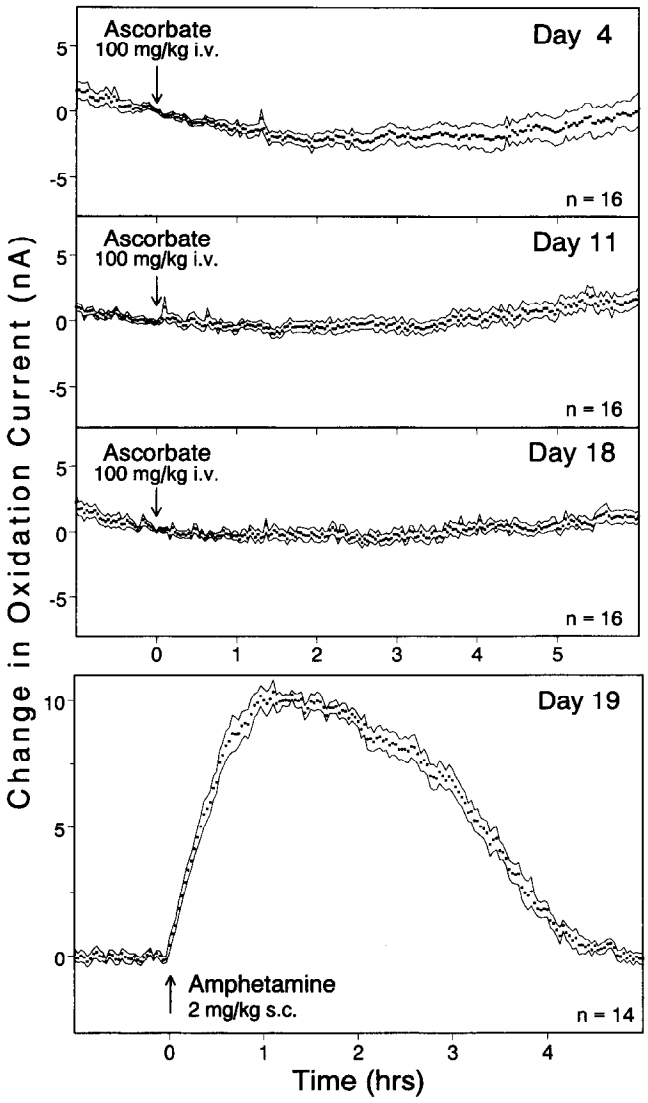

FIG. 6. Mean change in oxidation currents recorded in the striatum of freely moving rats (top panels) before and after administration of ascorbate (100 mg/kg in saline/heparin vehicle IV). Chronoamperometric recordings were conducted on days 4, 11, and 18 following chronic bilateral implantation of SGEs. Ascorbate responses did not differ significantly across all test days, $F(2, 45) = 1.61$, $p = 0.09$. Note that following these ascorbate treatments (bottom panel), administration of *d*-amphetamine (2 mg/kg SC) increased DA oxidation currents (+10.18 ± 0.53 nA) recorded from these electrodes on postimplantation day 19. These responses did not differ significantly from those shown in Fig. 4A, Intact, $F(1, 20) = 2.14$, $p = 0.17$. Symbols (■) and solid lines represent the mean chronoamperometric responses and SEM from 14–16 electrodes per treatment, respectively. For clarity, every second measurement taken is shown.

Moreover, the detection threshold corresponded to a $[DA]_e$ value of less than 19 ± 6 nM. Although the reverse-dialysis procedure cannot be used to provide an accurate estimate of basal DA concentrations in the ECF, as detected by SGEs, extrapolation from $[DA]_e$ values suggests that these concentrations in the striatum would be significantly less than 86 nM. Estimates provided by microdialysis no-net-flux procedures have been reported in the range of 10–15 nM (56).

The third criterion maintains that the electrochemical signals detected in vivo must reflect on-going neuronal impulse activity. It is well known that high systemic doses of γ-butyrolactone abolish completely the firing activity of DA neurons (46,61), an effect that has been shown by microdialysis to be accompanied by dramatic long-term decreases in DA and DOPAC efflux in the striatum (~8-h duration) (35). Dopaminergic activity, along with basal DA efflux in the striatum, can also be completely disrupted for long durations (>2 h) by medial forebrain bundle perfusion with TTX (45). Thus, it is highly significant that the time course and magnitude of decreases in the baseline chronoamperometric signal induced by γ-butyrolactone and TTX were very similar to previous findings using microdialysis (35,45), suggesting overall, that the baseline chronoamperometric signal is dependent upon continuous DA neuronal activity and reflects moment-to-moment changes in the basal activity of DA in the ECF.

The fourth criterion that SGEs monitor basal DA efflux derived from intact and functional dopaminergic terminals in the striatum was fulfilled in the present studies by showing that 6-OHDA lesions induced a marked attenuation in *d*-amphetamine's facilitatory effects on the chronoamperometric baseline signal, and also caused a marked reduction in the inhibitory effects of γ-butyrolactone. These latter findings with γ-butyrolactone are significant in a number of respects, as they suggest that 6-OHDA lesions significantly decreased normal basal levels of DA by at least 90%, as noted by a γ-butyrolactone–induced decrease of 1 nA in lesioned rats as compared to a 10 nA decrease in intact rats (see Fig. 4B). These results are consistent with recent microdialysis and 6-OHDA lesion studies showing an 80% attenuation of DA efflux by *d*-amphetamine and corresponding decrease in basal dialysate DA concentrations of ~85% (14,53).

A final criterion requires that responses measured by SGEs in the striatum conform with the known pharmacology of DA neuronal systems, and that these effects can be replicated after prolonged implantation of the probes in brain tissue. A number of microdialysis studies (34,40,41,54,55,65) have reported increases in striatal DA efflux following administration of *d*-amphetamine or nomifensine based on the ability of these drugs to facilitate DA transmission by accelerated exchange diffusion (25) and blockade of the DA uptake transporter (16), respectively. The effects of *d*-amphetamine and nomifensine on the chronoamperometric signal recorded over the 18–19 day test period were entirely consistent with the actions of these drugs and were comparable in pattern and magnitude of change observed using voltammetry and carbon fiber electrodes in the striatum of awake hamsters and rats (26,32). However, the present *d*-amphetamine results differ in a number of respects with those reported using microdialysis. For example, dialysis studies using similar doses of *d*-amphetamine typically report a latency of ~40 min to reach peak effects, with peak amplitudes ranging from 10- to 40-fold of baseline DA. In the present study, *d*-amphetamine induced only a twofold increase in the signal above baseline levels, based on the SGE estimate of an average basal oxidation current of ~10 nA in the striatum. These discrepancies in magnitude and time course measures of DA efflux may be related to differences in the ECF pool sampled by SGEs and, in particular, may reflect a drainage effect of microdialysis and its impact on steady-state concentrations of neurochemicals in the surrounding ECF compartment (3,5,33). Although these explanations remain speculative at this time, several studies using in vivo electrochemical techniques have reported similar time course and magnitude-related differences in mesostriatal

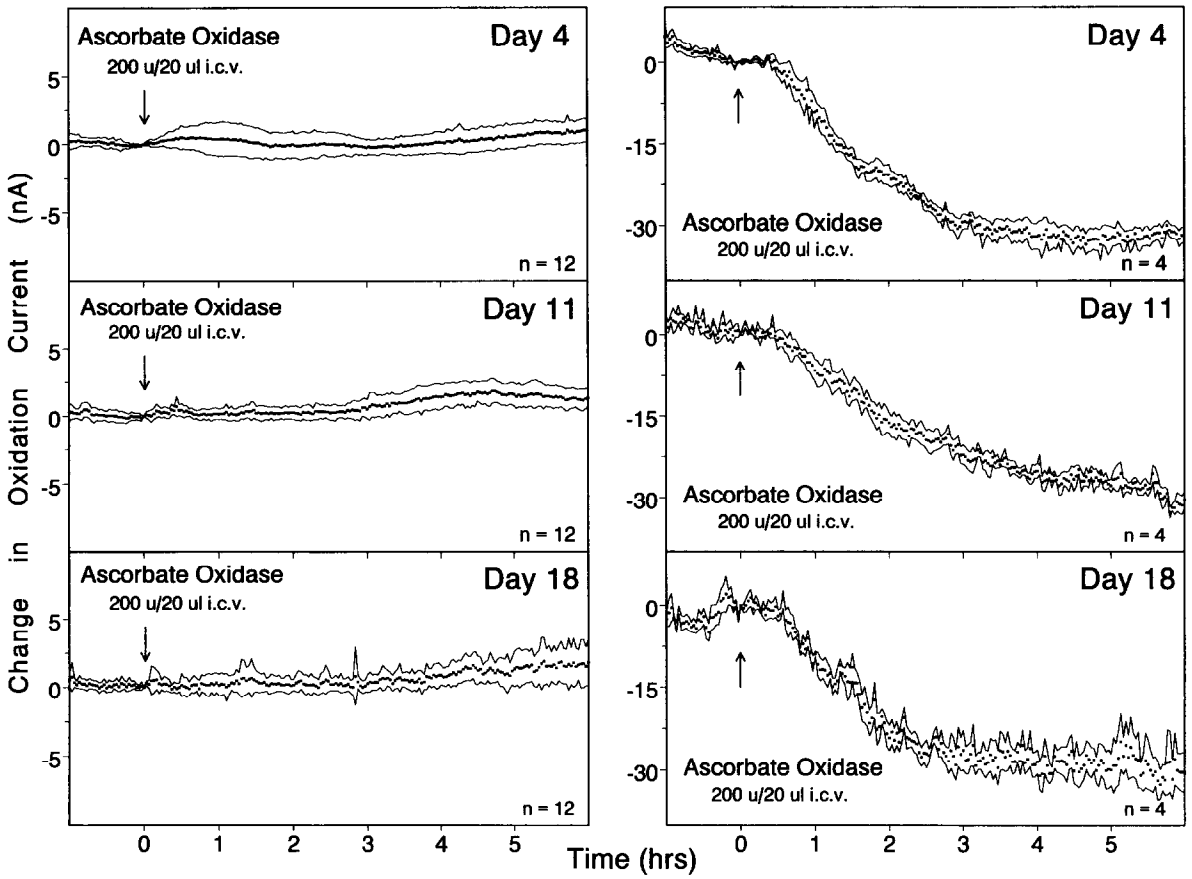

FIG. 7. Mean change in oxidation currents recorded from chronically implanted (left panels) SGEs and (right panels) CGEs in the striatum of freely moving rats before and after intraventricular (ICV) administration of AA-oxidase (200 units/10 μl/10 min). Chronoamperometric recordings taken from chronically implanted CGEs in separate groups of rats show mean AA-oxidase–induced decreases of −32 ± 3, −28 ± 2, and −31 ± 3 nA on days 4, 11, and 18, respectively. Symbols (■) and solid lines represent the mean chronoamperometric responses and SEM from 4–12 electrodes per treatment, respectively. Chronoamperometric responses to AA-oxidase did not differ significantly across all test days for SGEs, $F(2, 33) = 1.44$, $p = 0.22$, or CGEs, $F(2, 9) = 1.89$, $p = 0.12$. For clarity, every second measurement taken is shown.

DA efflux with psychomotor stimulants, including opiates, compared to those reported with microdialysis (20,37,38,43).

As expected, repeated administration of γ-butyrolactone elicited reproducible temporal patterns of decrease (−80%, 4-h duration) in the chronoamperometric baseline signal over the 18-day test period. These effects also were in agreement with recent voltammetry results (22), and differed only in minor respects with microdialysis results (−55%, 3-h duration) with γ-butyrolactone (36). It is worth noting that γ-butyrolactone at higher doses decreases basal ECF levels of DOPAC. However, at the present dose, DOPAC remains unaffected (36) providing further support that the observed decrease in the chronoamperometric signal could not have been related to DOPAC. Rather, together with the actions of *d*-amphetamine and nomifensine, these longitudinal studies indicate that SGEs remain viable over long-term periods of implantation in tissue and provide highly reproducible data resulting from pharmacologically induced inhibition or facilitation in the efflux of DA from synaptic terminals in the striatum.

Although, as noted above, side-by-side reverse dialysis of various compounds, including AA and DOPAC, provided some of the most convincing in vivo evidence in support of SGE selectivity to DA, the time-dependent loss in the efficiency of microdialysis probes precluded their use as a test of SGE selectivity beyond a 2–3-day implantation period. Therefore, long-term selectivity of SGEs was examined by monitoring the effects of systemic injections of AA, pargyline, and ICV infusions of AA-oxidase on the chronoamperometric signal over the 18-day test period. With respect to AA, several electrochemical studies utilizing carbon fiber and CGEs (7,17,23,47) have shown that systemic administration of AA increases by two- to threefold endogenous levels of AA in the striatum. In this respect, the failure of injections of AA to modify the chronoamperometric baseline signal over 18 days of implantation is consistent with our reverse dialysis results with AA and serves to indicate that SGEs remain insensitive to μM increases in AA over extended periods of contact with brain tissue. These data however, do not address the issue of an indirect contribution of AA to the chronoamperometric signal via AA's electrocatalytic effects (6). Under in vivo conditions AA electrocatalytic mechanisms may be saturated and accordingly, if AA were contributing to the SGE baseline signal in this manner only marked decreases in ECF AA would have any consequential effect. In the present

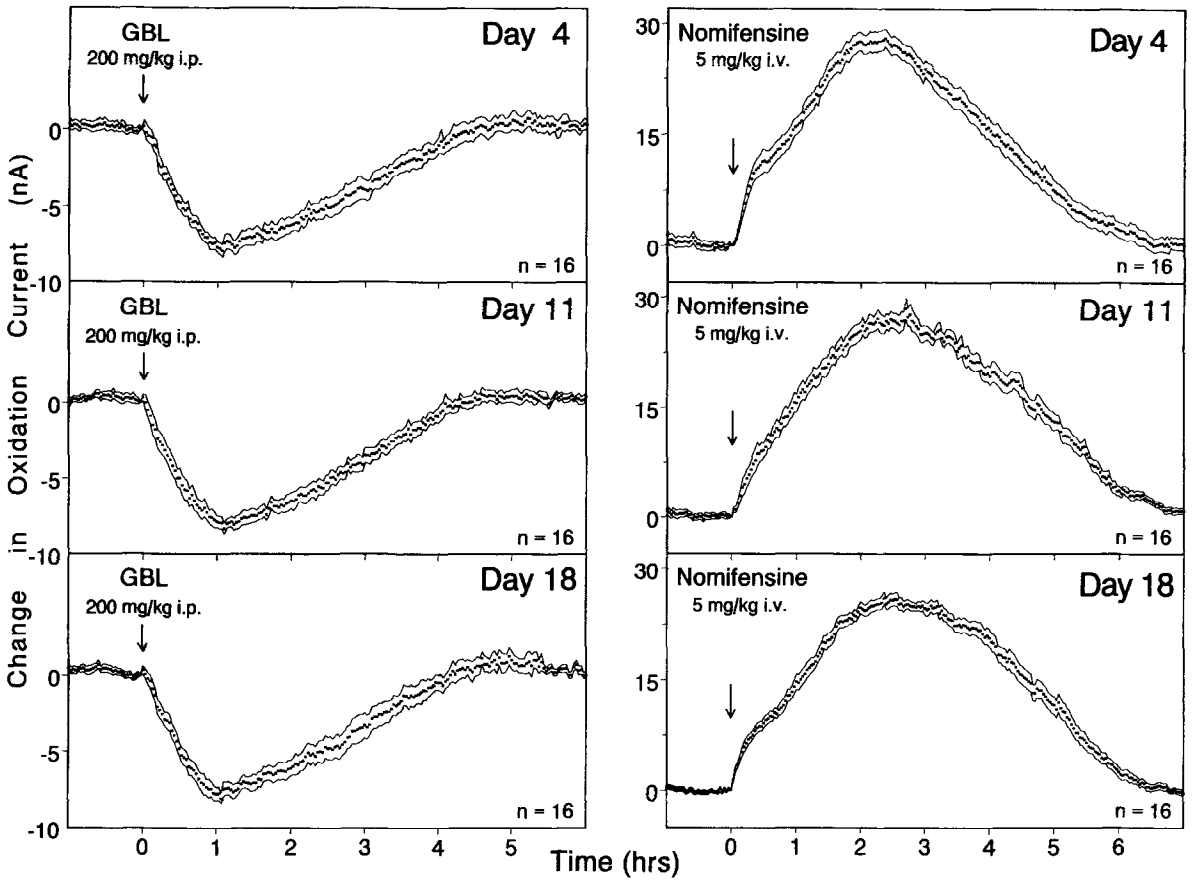

FIG. 8. Mean change in oxidation currents recorded in the striatum of freely moving rats before and after administration of (left panels) γ-butyrolactone (GBL, 200 mg/kg IP) and (right panels) nomifensine (5 mg/kg in saline/heparin vehicle IV). Mean changes in baseline oxidation currents induced by γ-butyrolactone and nomifensine corresponded to −7.97 ± 0.42, −8.32 ± 0.4, and −8.1 ± 0.32 nA, and +27.6 ± 3, +26.5 ± 2, and +25.8 ± 1 nA, on postimplantation days 4, 11, and 18, respectively. Symbols (■) and solid lines represent the mean chronoamperometric responses and SEM from 16 electrodes per treatment, respectively. Chronoamperometric responses did not differ significantly across all test days for γ-butyrolactone, $F(2, 45) = 0.59$, $p = 0.56$, or nomifensine, $F(2, 45) = 1.78$, $p = 0.19$. For clarity, every second measurement taken is shown.

experiments, ICV infusions of AA-oxidase, at doses that produce extensive long-lasting decreases in AA levels without affecting levels of DA or DOPAC (8,12), failed to modify the chronoamperometric signal over 18 days of testing. As such, these data represent the most convincing evidence in support of the long-term insensitivity of SGEs to AA in brain tissue. This property of SGEs may involve the unique interaction of the SGE surface with certain auto-oxidation inhibitor macromolecules associated with albumin-like proteins found in the soluble fraction of rat brain tissue (10).

Repeated administration of pargyline also failed to significantly alter the baseline chronoamperometric signal over the 18-day test period, suggesting that SGEs also remain unresponsive to changes in ECF levels of DOPAC. These findings, however, also point to important differences with respect to results obtained using microdialysis and MAO inhibitors, as a number of dialysis studies report marked elevations in ECF DA concentrations following systemic injections of pargyline (35,55). Accordingly, the present results suggest that SGEs may be responding to a distinctly different ECF pool of DA that may be physically unperturbed. Recent microdialysis studies indicate that pargyline-induced expression of c-*fos* occurs only in striatal tissue adjacent to the dialysis probe and not at a distance greater than 600 μm from the probe (19,44). While the mechanisms underlying these localized effects of pargyline remain to be specified, these investigators have postulated that they are a result of an interaction between an altered function of DA terminals related to the presence of the dialysis probe in tissue, in combination with pargyline-induced increases in the intracellular levels of DA available for release into the ECF. These data have, therefore, raised the possibility that MAO inhibition by itself does not cause an increase in ECF DA in intact unperturbed striatal tissue (19,44). This, in turn, may explain the fact that pargyline is not associated with increased motor activity, as is the case with other drugs that increase DA in the ECF (e.g., *d*-amphetamine) (41). Preliminary experiments in our laboratory agree that the DA-enhancing effects of pargyline may be related to interactions of the dialysis probe with tissue. A pargyline-induced increase in the chronoamperometric signal, indistinguishable from a typical pattern recorded by microdialysis, was observed with SGEs chronically implanted adjacent to a microdialysis probe in rat striatum.

The present observations of a complete lack of effect of

enzymatic elimination of AA, systemic injections of AA and pargyline, and AA/DOPAC or 5-HT/5-HIAA reverse-dialysis treatments on the monitored chronoamperometric baseline signal recorded from SGEs at +0.2 V, strongly suggest that these signals do not reflect changes in AA, DOPAC, or indole concentrations in brain ECF. Rather these data, combined with the pharmacological responses to dopaminergic agents and other treatments, and our previous studies on evoked efflux of DA by electrical stimulation of the ventral tegmental area (9), are consistent with the hypothesis that the chronoamperometric signals recorded from chronically implanted SGEs represent changes in DA efflux from a pool of functionally intact DA nerve terminals. Overall, these studies provide further evidence that the SGE can serve as a valid biosensor to study DA neurotransmission in the CNS with the added advantage of being applicable to chronic within-subject investigations of DA neurochemical correlates of behavior (20,49–51).

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