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LOCAL DEPLETION OF MONOAMINES INDUCED WITH *IN VIVO* VOLTAMMETRY IN THE CAT BRAIN

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Abstract—A significant depletion of the electroactive monoamines and their metabolites in the vicinity of a carbon fiber microelectrode may be induced by *in vivo* staircase voltammetry in the brain, even if the duration of the voltammetric scans is relatively short (≈5 s). The variation of this depletion was determined in the extracellular fluid of the cat thalamus at different durations of the pauses separating consecutive measurements. Pauses not shorter than 5 min ensured a nearly full relaxation, so that at the beginning of each subsequent scan a virtually undisturbed environment surrounded the electrode. With pauses shorter than 5 min, it is still possible to monitor major changes in the monoamine concentration. Staircase scans separated with 45 s pauses, for example, were suitable to study the increase in monoamine levels after administration of reserpine, and release phenomena stimulated with KCl were monitored with frequently repeated voltammetric pulses. The electrochemically induced depletion, on the other hand, can be used for characterizing the dynamics of mass transport in the studied brain structure. This was demonstrated with staircase voltammetry alternated with pauses of 1–100 s, and with quasi-chronoamperometry.

In vivo brain voltammetry is generally used for monitoring extracellular monoamine (including dopamine) levels. These may be significantly altered by the voltammetric measurement itself through depletion in the vicinity of the electrode. This effect can be minimized with appropriate selection of sampling intervals and other parameters of staircase voltammetry. Conversely, depletion and the following relaxation can be used for determining dynamic characteristics of the studied brain structure which would be difficult to obtain otherwise.

Voltammetric carbon fiber microelectrodes have been successfully used for monitoring the concentration level of monoamines (including dopamine) and their metabolites in the extracellular fluid of living animal brain.[3,5,8,10] Electrochemical pretreatment[6,7] increases the sensitivity of these electrodes for catechol- and indoleamines with respect to ascorbate. Application of a thin Nafion coating[2] enhances the selectivity for dopamine with respect to both ascorbate and neurotransmitter metabolites. With different pulse techniques monoamine levels were monitored[3] and phenomena related to stimulated neurotransmitter release have been detected.[5,8,10] Cyclic voltammetry can be performed at such a high speed (300 V/s)[13] that a subsecond temporal resolution of release and uptake becomes possible.[11] The large capacitive background currents, however, need to be subtracted numerically using this technique.

In staircase voltammetry Faradaic currents are predominant, and a somewhat better chemical resolution can be ensured. These advantages are basically due to the much lower scan rate, which implies a poorer time resolution and a longer duration of the measurement (≈5 s or longer). As a consequence, a significant depletion of the electroactive species is induced in the vicinity of the working electrode. This effect is often severe, despite the fact that mass transport is generally facilitated by the use of a microelectrode.

In this work the extent of this depletion has been determined with carbon fiber microelectrodes *in vivo* in the thalamus of the cat brain, as a function of the duration of the delay between subsequent staircase scans. The data were used for determining the minimal duration of the delays, which is already long enough to allow for perfect relaxation of the depleted layers, so that each subsequent scan reflects a virtually undisturbed environment. On the other hand, the electrochemically induced depletion (and the following relaxation) can be used for characterizing the dynamics of mass transport of the electroactive species in the studied brain structure. To demonstrate this possibility, repetitive staircase voltammetry, repetitive pulse voltammetry and quasi-chronoamperometric experiments have been performed.

This report complements previous studies of electrochemical depletion and local concentration dynamics by Cheng *et al.*,[4] Justice *et al.*[9] and others, whose work involved a different technique (e.g.

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Abbreviations: DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanilic acid; VPL, ventro-postero-lateral thalamic nuclei.

repetitive pulse chronoamperometry at a carbon disk electrode[4]) and/or different brain compartments.[9] The major unique objectives of this study were (1) optimizing the repetition rate of *in vivo* voltammetry and (2) measuring the dynamics of concentration recovery after electrochemical depletion. The reasons for choosing the thalamus of cat were: (1) relatively large voltammetric signals could be obtained, and (2) the larger dimensions facilitated electrode positioning with respect to major dopaminergic brain structures in the rat.

EXPERIMENTAL PROCEDURES

The pyrolithic carbon fiber microelectrode (35 μm, AVCO, U.S.A.) was fixed into a glass micropipet with epoxy resin so that its active length was $\approx 200\ \mu$m. Mercury and a stainless steel wire provided the electrical contact. After electrochemical pretreatment[6,7] it was slowly inserted into the ventro-postero-lateral thalamic nuclei (VPL, A: 8–10; L: 6; V: 5–7[1]) of cats anesthetized with Chloralose (50 mg/kg, i.v.). This was done in 0.5 mm steps interrupted with 10 min breaks for mechanical relaxation of the adjacent brain structure. The carbon fiber electrode, an Ag/AgCl microreference electrode and a stainless steel wire as auxiliary electrode were connected to a potentiostat that was controlled with a TPA-i computer via a fast CAMAC interface (both made by KFKI, Hungary). During measurements the rectal temperature was kept constant with a heating pad, and seven to eight individual animals of both sexes were used in each experiment except for the reserpine and KCl experiments, where three to four animals were used.

Calibration was performed before and after each series of measurements *in vitro*, with respect to 5-hydroxyindoleacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC), and ascorbic acid in 0.9% saline solutions buffered at pH 7.4. The calibration graphs were non-linear but still monotonic at low concentrations ($<2\ \mu$M). Decreased sensitivities were obtained after *in vivo* application. The observed time-dependent decay in the voltammetric signal with staircase voltammetry was not related to this finding, because after a longer pause (≥ 5 min) during *in vivo* experiments, the original signal level always recovered. Thus, the electrode sensitivities must have decreased soon after implantation, during the first 3–4 *in vivo* test scans, the results of which were always discarded. The selectivities were tested with a solution containing all three substances (peaks: ascorbic acid -20 mV; DOPAC $+100$ mV; 5-HIAA $+290$ mV).

Staircase voltammograms were obtained by scanning from -200 to $+600$ mV with 10 mV/60 ms steps. Thus, the duration of one scan was 4.8 s. During the final 10 ms of each step, 50 current samples were measured and averaged by the computer, to increase the signal to noise ratio. The smoothed first derivative of the voltammograms was used for evaluation. To monitor K^+-evoked release, 100 ms pulses of $+600$ mV were alternated with 100 ms intervals at 0 mV applied voltage. The current was sampled during the last 10 ms of the pulses the same way as with staircase voltammetry. The quasi-chronoamperometric experiments consisted of two subsequent periods. During the first 10 s fixed potential amperometry was performed at $+600$ mV. After a steady depletion had been established, the oxidation was stopped, and the current was measured at $+600$ mV during 10 ms pulses separated with 0.5–2 s intervals. In one set of experiments 0 V was applied during these intervals, and in another set the cell was open-circuited between subsequent sampling periods. During the pulses, 50 current samples were averaged. These pulses were short enough with respect to the intervals, so that the relaxation was monitored while being virtually undisturbed by the electrolysis going on during sampling.

RESULTS

Minimizing depletion at repetitive staircase voltammetry

The first derivative of the *in vivo* staircase voltammograms exhibits a peak around $+300$ mV. As *in vitro* experiments indicated, 5-HIAA, and probably homovanilic acid (HVA), contribute to this peak. Its area is correlated with the total concentration of the electroactive species in the vicinity of the electrode. This concentration apparently decreases if the scans are repeated at a relatively high frequency. This can be clearly seen in the series of measurements shown in Fig. 1, where 4.8 s scans and 3 s pauses were alternated. This decrease in the integral is even more predominant if a shorter pause (2 or 1 s) is used. When longer pauses are selected then the effect becomes less significant: in the case of, e.g. 4.8 s scans and 100 s pauses, an almost negligible decrease is observed (Fig. 2).

It is evident from these tendencies (and from their reproducibility) that the most important factor contributing to the signal decrease is the depletion of the

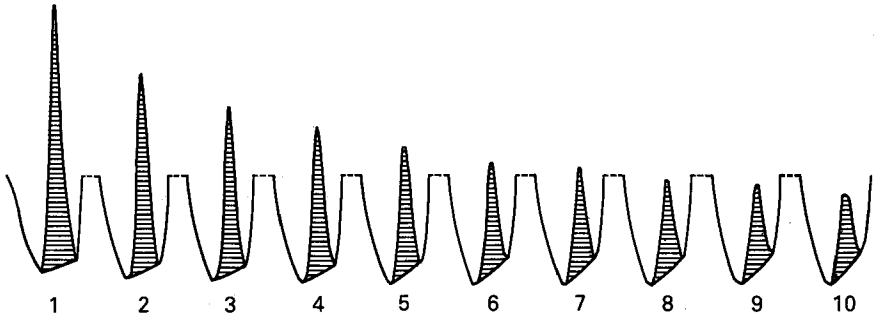

Fig. 1. Smoothed first derivative of experimental staircase voltammograms obtained *in vivo*. Pauses (waiting periods at -200 mV) of 3 s were alternated with scans of 4.8 s. The serial number of subsequent scans is indicated below each curve. Before scan no. 1 no measurement has been made for at least 30 min (the cell was disconnected). Excerpts of the curves only are shown, with the peak at about $+300$ mV. Peak area, which is the peak integral above a linear baseline, is shaded.

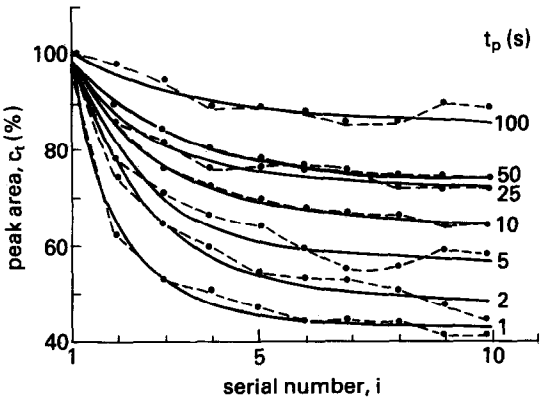

Fig. 2. Dynamic depletion curves obtained *in vivo* at different measuring frequencies. Each point corresponds to a peak area similar to those shown in Fig. 1, and each individual curve is characterized by a fixed duration of pause, t_p, separating subsequent scans. Dashed lines connect points measured at identical pauses in a series. Solid lines are exponential curves according to Eq. (1), fitted to the measured points (peak areas) with the criterion of least squares. The residual error of the fittings was 0.5–2.8%. The points measured first in any series are considered as 100%.

electroactive species, which is caused by the electrolysis during the repeated scans. In other words, the composition of the extracellular fluid is disturbed by its intended measurement. Similar findings have also been described when techniques other than staircase voltammetry and other types of electrode were used.[4] The extent of this adverse effect can be minimized in two ways. (1) The scans can be performed faster. Then the contribution of capacitive currents becomes larger, causing a deterioration of the signal/background ratio. (2) Scans can be repeated less frequently. In this case the dynamics in concentration changes that can be monitored with voltammetry become more sluggish. Thus, in any practical case a compromise must be found between the constraints of the measurement and the kinetics of the monitored process.

In some cases it may be important to ensure full relaxation during each pause, so that every scan reflects the studied extracellular fluid compartment virtually undisturbed. To derive the minimal duration of pauses satisfying this condition in the cat VPL, a three parametric exponential function,

$$c_t = \alpha + \beta \exp[\gamma(i-1)], \quad (1)$$

has been fitted to the measured peak areas with a least squares criterion at any given duration of pause (solid lines in Fig. 2). Here c_t is the peak integral corrected for a linear background line. This variable is proportional to the total concentration of the electroactive species, as measured by the carbon fiber microelectrode. Its value is considered to be 100% in the first scan. The serial number of consecutive voltammograms is i, and the parameters α, β and γ characterize the dynamics of increase in depletion at a fixed repetition rate. The final steady depletion, D_∞, can then be defined as follows:

$$D_\infty = \beta/(\alpha + \beta), \quad (2)$$

which was found to depend on the duration of the pauses according to a logarithmical relationship, as shown in Fig. 3:

$$D_\infty = D_1 - S \log t_p, \quad (3)$$

where t_p is the duration of the pauses between subsequent voltammetric scans and D_1 is the depletion observed when pauses of 1 s have been applied (log means logarithm of 10). Under the given experimental circumstances (cat VPL, 4.8 s scans from -200 to $+600$ mV at a 35×200 μm carbon fiber), $D_1 = 0.56 \pm 0.01$ and $S = 0.23 \pm 0.02$ have been found by averaging the results of three different sets of experiments, each performed with a different individual electrode.

Though strictly speaking Eq. (3) with the above parameter values is valid only in the 1–100 s pause range, it is worthwhile using it for determining the minimal duration of pauses, t_{relax}, which formally results in zero (steady) depletion:

$$t_{relax} = 10^{D_1/S}. \quad (4)$$

In the studied case, we have found that $t_{relax} \approx 5$ min (see the intersection of the straight line with the $D_\infty = 0$ axis in Fig. 3). Physiologically, this result means that all concentration disturbances caused by the applied scans disappeared in about 5 min. In other words, if the consecutive scans are separated with pauses of at least 5 min, then at the beginning of

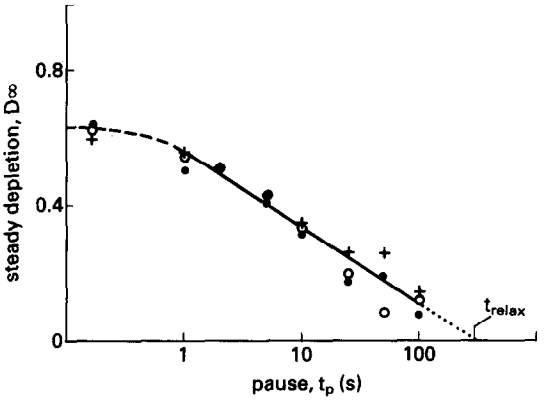

Fig. 3. The final steady depletion as defined by Eq. (2), as a function of the duration of pauses. The horizontal axis is logarithmically scaled. Results of three sets of experiments done with three different individual electrodes in different animals are shown [(+) 1; (○) 2; (●) 3 electrode]. The solid line was obtained with a linear regression using the data of all three electrodes in the pause range 1–100 s. In this regression $r^2 = 0.94$. For the separate data of the individual electrodes, $r^2 = 0.96(1)$, $r^2 = 0.96(2)$ and $r^2 = 0.98$ (3). The points measured in the range of the dashed line were not used in calculating the regression. The dotted line illustrates the extrapolation according to Eq. (3), which results in the formal relaxation time being characteristic of the studied brain structure, t_{relax} [Eq. (4)].

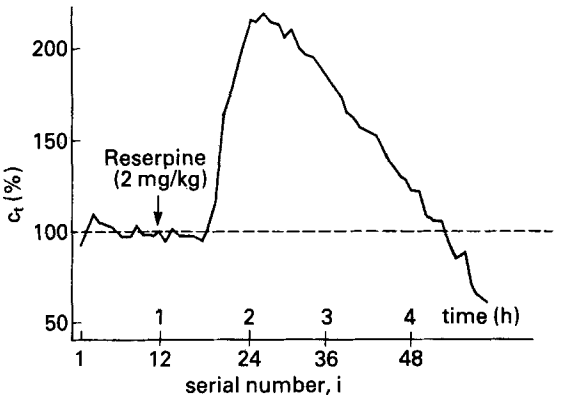

Fig. 4. Reserpine-induced release as monitored with the area of the staircase voltammetric peak around +300 mV ($E_{1/2} = +335 \pm 12$ mV). The repetition rate was 5 min, and the duration of each scan was about 5 s. The arrow indicates the time of reserpine injection.

each measurement, the extracellular fluid is fully relaxed, being virtually undisturbed by the measurement itself.

Trade-off between sampling rate and depletion

There are monoamine changes that are slow enough so that they can be monitored with a sufficient temporal resolution and still signal depletion is avoided. Such is the effect of reserpine, a massive re-uptake inhibitor. Figure 4 displays the transient obtained after drug administration (2 mg/kg, i.v.). The 5 min repetition rate (=almost 5 min waiting periods) ensured virtually full recovery between subsequent measurements. The experiments were started 1 h before and terminated 3 h after drug injection. The induced increase in the +300 mV peak reached its maximum after about 1.5 h.

When only trends or major changes are to be monitored instead of undisturbed monoamine levels, it is possible to use a repetition rate that is higher than the one which would ensure full relaxation. The above effect has also been successfully reproduced with the alternation of scans of about 5 s and pauses of 45 s (in another animal). The resulting curve was, however, depressed by a steady depletion of about 20%, as a consequence of the more frequent scanning [Eq. (3) and $t_p = 45$ s yield $D_\infty = 0.18$].

Contrary to the effect of reserpine, it is often necessary to accept a certain level of steady depletion. As an example, K^+-evoked releases have been monitored with a repetitive pulse technique that must have been causing a depletion even more severe than that encountered previously with staircase voltammograms (measured at every 50 s). However, this affected only the baseline (Fig. 5), while the induced current excess revealed relevant information: its area was proportional to the amount of the applied KCl. The slopes of both the increasing and relaxing excess signal remained independent of this amount. Both sides were nearly linear, and the slope of the increase was about six to eight times larger than that of the following decrease.

It must be noted that while the area of any staircase voltammetric peak is selectively related to the concentration of one or a few substances, this latter technique involves the oxidation of all components that are electroactive at and below +600 mV. So, the K^+-evoked transient current non-selectively reflects

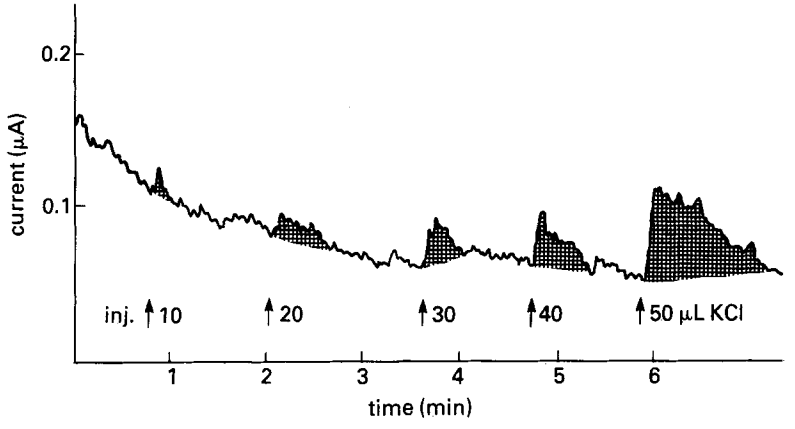

Fig. 5. K^+-evoked release as monitored with pulses at +600 mV. Arrows show instance of KCl ejection by a microsyringe. The tip of the infusion capillary was placed at a distance of ≈1 mm from the working electrode in the thalamus.

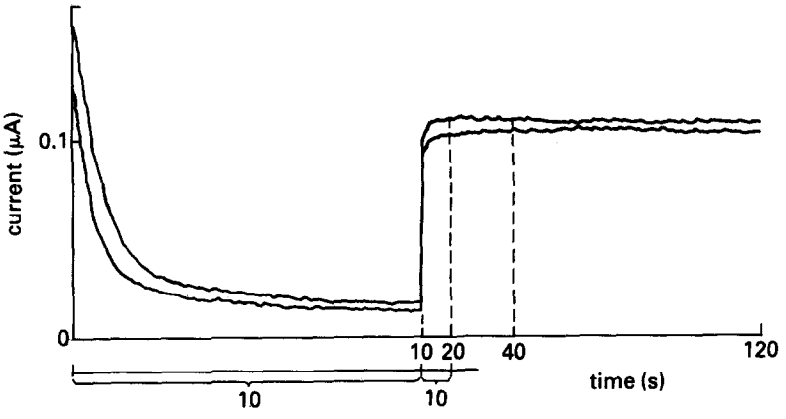

Fig. 6. Depletion and following relaxation as monitored with quasi-chronoamperometry at +600 mV. Depletion and relaxation are shown with different time scales. The results of two experiments are displayed to characterize the degree of reproducibility.

changes in the concentrations of 5-HIAA, HVA, DOPAC and possibly other substances.

Characterization of the dynamics of depletion and relaxation with staircase voltammetry and with quasi-chronoamperometry

Scanning under the discussed circumstances more frequently than once every 5 min can be useful, in fact, for two purposes: besides monitoring trends and major changes (see above), information about the dynamics of depletion and relaxation in the studied brain structure can be obtained. Such information is involved in the curves shown in Fig. 1, and more specifically in Fig. 2. Apparently, a steady state was established after a few scans in all sets of experiment. It is interesting that the fitted values of parameter γ in Eq. (1) were found to be similar for every studied measuring frequency, despite the large variation in the time scale of the experiments. On average, $\gamma \approx -0.3$, or in other words, three to four consecutive scans were sufficient to induce half of the final steady depletion. The average "half-life time" of the depletion process was 3.3 scans, which was nearly independent of the duration of pauses in a broad range ($1\text{ s} \leq t_p \leq 100\text{ s}$). This characteristic, as well as α and β, depends on both the parameters of the applied scans (potential range, duration, step size) and the dynamic properties of the studied brain structure. The relaxation time, t_{relax}, has a clear physical meaning: it represents the time necessary for complete recovery of the local concentrations to the pre-perturbation levels. Consequently, it is less dependent on the characteristics of staircase voltammetry than the other parameters in Eqs (1)–(3). However, t_{relax} is the result of an extrapolation and thus its statistical reliability is relatively poor. The monoamine (and metabolite) transport can also be characterized with the steady depletion obtained at, for example, pauses of 10 s: $D_{10} \approx 0.33$ can be calculated with our data. This value can be measured faster and obtained without extrapolation, but its strong dependence on the scanning parameters is evident.

In spite of the clear exponential character of the depletion curves (Fig. 2), repetitive staircase voltammetry is not appropriate for developing and testing a more detailed dynamic model of monoamine transport, because the technique represents a complicated perturbation: depletion and relaxation are alternated, and during every scan the depletion is changing as a function of voltage. A more unequivocal concentration perturbation of the extracellular compartment can be induced when a constant voltage is applied for a longer period of time, and the current decay is monitored simultaneously. After reaching steady state, the voltage can be either reduced or disconnected, and the concentration relaxation can be followed with short pulses at the same voltage that was used previously for depletion. Two depletion–relaxation curves obtained in such a way are shown in Fig. 6, being characteristic of the VPL of the cat thalamus. The voltage used for depletion was +600 mV, while at relaxation the working electrode was disconnected.

The decay in current corresponds to the depletion of all chemical species that are electroactive at or below +600 mV. The depletion again exhibited a final steady state, with an extremely low steady level ($\approx 11\%$ of starting current) and a "half-life time" of about 0.5–1 s. We tried simple functions to fit the decay, with the exponential function giving the best fit (Table 1). None of the functions were suitable to

Table 1. Statistical quality of fitting simple functions to depletion and relaxation curves obtained with quasi-chronoamperometry

Function type	Residual S.D. (%) Depletion	Relaxation
$A + B\exp(Ct)$	1.7	5.4
$A + B/\sqrt{t}$	5.6	6.2
$A + B/t$	2.7	5.4
$A + B/t^2$	2.0	5.6

fit the relaxation curves. Interestingly, when 0 mV was applied to the carbon fiber electrode instead of being disconnected, the relaxation exhibited a clear 1–2% overshoot and thus no monotonic function was appropriate for fitting. When during relaxation the cell was open-circuited, no overshoot could be observed.

DISCUSSION

Complementing the results of others, this study demonstrates that repetitive staircase voltammetry may cause a significant depletion in the electroactive species in the extracellular compartment adjacent to the carbon fiber microelectrode. Consequently, the extracellular fluid is not undisturbed even at the beginning of the scans if the pauses between subsequent measurements are shorter than the typical relaxation time of the studied brain structure. In the VPL of the cat thalamus, this time is about 5 min for the +300 mV peak studied. It must be noted, however, that the relaxation time as defined in this work is not fully independent of the scan and electrode parameters. It may also vary in other structures of the cat brain, and/or in animals other than cat (the extent of this variation remains a subject of further research).

An *in vitro* calibration cannot be used for the quantitative evaluation of individual voltammograms in most cases for this reason alone. There is another problem associated with concentration measurements: at constant potential electrolysis, about 0.5–1 s was sufficient for the current to drop by half of its total (final) decrease, the latter being almost 90% of the initial current. Thus, the total change amounted to an order of magnitude (Fig. 6)! So, local depletion is a rapidly advancing and very significant process in the brain. This is a consequence of the special geometry of the extracellular fluid, which can be visualized as a complex network of channnels characterized with a low volume fraction (≈ 0.2) and a high tortuosity (≈ 1.6).[12] This situation is hard to match with any *in vitro* calibration arrangement. Another consequence of this geometry is that, conversely, relaxation is a rather slow process. In the VPL of the cat thalamus we obtained relaxation times of the order of 5 min. Fast depletion and slow relaxation imply that even when the consecutive staircase voltammograms are separated by at least 5 min pauses, depletion develops within 1 s and so only the beginning of the voltammograms represent an undisturbed environment.

As a viable compromise between minimizing depletion and maximizing temporal resolution, trends instead of undisturbed concentrations can be monitored even if the background current is biased by some depletion. This was demonstrated by monitoring reserpine-induced changes with staircase voltammetry, and K^+-evoked release with a repetitive pulse technique. These considerations may also favor differential pulse[3,10] or fast scanning techniques,[11,13] despite the large capacitive currents associated with them. A Nafion-coated electrode[2] contributes an additional mass transport barrier which should further enhance depletion effects. With short electrolysis times, depletion may be confined predominantly within the coating. Thus, the use of Nafion-coated electrodes may be advantageous despite the more depressed signals, because local depletion during *in vitro* calibration and *in vivo* measurements are more similar than at bare electrodes. Increased selectivity for dopamine[2] is a further advantage.

The relaxation time obtained with staircase voltammetry is characteristic of both the brain structure and the parameters of the scanning (though it depends on the scan parameters to a lesser extent than the other introduced kinetic parameters). The dynamics of depletion and concentration recovery of monoamines (and metabolites) can be studied in a more straightforward way with quasi-chronoamperometry, where the resulting current–time curves are characteristic of the studied structure only. The observed overshoot in the relaxation curves may be a sign of the existence of a biological feedback loop that "acts" in order to compensate for the depleted monoamines. Simple mathematical functions cannot be fitted to either the depletion or relaxation current.

Derivation of more detailed mathematical models have been reported for different techniques and structures[4,9] other than those studied in this work. We believe that the experimental approach reported here may contribute to the identification of some structure-specific transport parameters, an example of which is the relaxation time as derived with the analysis of staircase voltammetric results. With simple chronoamperometric experiments transport processes such as diffusion, release and uptake, convection and, possibly, some feedback regulatory circle could be studied in the extracellular fluid. Despite the use of a microelectrode, significant depletion was observed in all measurements, which may be related to the tortuosity of the extracellular transport pathways.

We intended to emphasize in this study that a single working electrode can be used for both perturbing the total monoamine concentration and monitoring the results of this perturbation. We note that irreversible adsorption onto and aging of the surface of the carbon fiber microelectrode were neglected. Instead of enhancing selectivity, rather the dynamics of the total monoamine (and metabolite) concentration were investigated.

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