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In Vivo Ambient Serotonin Measurements at Carbon-Fiber Microelectrodes

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Abstract

The mechanisms that control extracellular serotonin levels in vivo are not well-defined. This shortcoming makes it very challenging to diagnose and treat the many psychiatric disorders in which serotonin is implicated. Fast-scan cyclic voltammetry (FSCV) can measure rapid serotonin release and reuptake events but cannot report critically important ambient serotonin levels. In this Article, we use fast-scan controlled adsorption voltammetry (FSCAV), to measure serotonin's steady-state, extracellular chemistry. We characterize the "Jackson" voltammetric waveform for FSCAV and show highly stable, selective, and sensitive ambient serotonin measurements in vitro. In vivo, we report basal serotonin levels in the CA2 region of the hippocampus as 64.9 ± 2.3 nM ($n = 15$ mice, weighted average $\pm$ standard error). We electrochemically and pharmacologically verify the selectivity of the serotonin signal. Finally, we develop a statistical model that incorporates the uncertainty in in vivo measurements, in addition to electrode variability, to more critically analyze the time course of pharmacological data. Our novel method is a uniquely powerful analysis tool that can provide deeper insights into the mechanisms that control serotonin's extracellular levels.

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Notes

The authors declare the following competing financial interest(s): Dr. Michael Heien declares an actual or potential financial conflict of interest and is co-founder/equity holder in Knowmad Technologies, a licensee of University of Arizona (UA) intellectual property used in this research. This relationship has been disclosed to the UA Institutional Review Committee and is managed by a Financial Conflict of Interest Management Plan.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.anal-chem.7b01257.

Detailed description of mathematical model, statistical parameters obtained from model, standard errors, and tests of significance of the coefficients (PDF)

Graphical Abstract

Dysfunctions of the serotonin system are thought to underlie numerous neuropsychiatric disorders, such as depression, anxiety, and schizophrenia.^{1–3} A better interpretation of serotonin neurochemistry is crucial for understanding the roles of this neurotransmitter but insight into serotonin's chemistry has been limited by the difficulty of *in vivo* chemical measurements. Serotonin is particularly challenging to detect electroanalytically *in vivo* because of an inauspicious combination of low extracellular concentrations and the propensity of serotonin and serotonin metabolites to foul electrodes.⁴

In 2009, we optimized fast-scan cyclic voltammetry (FSCV) for measurement of endogenous serotonin release and reuptake *in vivo* using carbon fiber microelectrodes (CFMs).⁵ Since then, we have uncovered various important aspects of serotonin neurochemistry. For example, evoked serotonin release is under much tighter regulation than dopamine (DA),⁶ being subject to prolonged autoreceptor control and multiple reuptake mechanisms.⁷ Furthermore, a single dose of a selective serotonin reuptake inhibitor (SSRI) rapidly alters serotonin neuro-chemistry;⁸ an important finding because a chronic SSRI regimen must often be followed for clinical therapy.²

While FSCV continues to provide important insights into the mechanisms that regulate extracellular *in vivo* serotonin, the method has limitations. In particular, because FSCV is background-subtracted, it only reports information about concentration changes. These changes allow us to probe serotonin release and reuptake, however it would be invaluable to also determine serotonin's ambient (steady-state, extracellular or basal) concentrations.

There are very few methods that can quantitatively measure extracellular serotonin concentrations. By far, the most commonly used method is microdialysis which reports extracellular serotonin levels in the lower nanomolar range.^{9–13} We thus sought to design a reliable and accurate method for measuring serotonin's ambient concentrations with our carbon fibers that we could couple to our FSCV measurements.

We recently made basal measurements of dopamine with fast-scan controlled-adsorption voltammetry (FSCAV), which exploits the adsorption capabilities of activated CFMs. The method is robust, selective, fast, and sensitive with the additional allure that CFMs measure from discrete brain localities¹⁴ where tissue damage is minimized.¹⁵ Our studies revealed a fundamental coaction between evoked and ambient dopamine.¹⁶ Here, we orient this method toward serotonin.

We find that unique FSCAV serotonin signals (that resemble FSCV responses) are stable during repeated recordings over 120 min in vitro. We confirm that the majority of interfering analytes are unlikely to contribute to the serotonin FSCAV signal, bar serotonin's major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) which gives a small signal at high concentrations. Subsequently we show that 5-HIAA is unlikely to contribute to the signal when at physiological concentrations. Next, our method is utilized to report ambient, in vivo serotonin levels in the mouse hippocampus as 64.9 ± 2.3 nM ($n = 15$ mice, weighted average $\pm$ standard error) using a functional linear statistical model that we developed based on electrode and in vivo variability. Using a pharmacological approach, the in vivo signal is validated and interferences by 5-HIAA, dopamine, and norepinephrine^{17–21} are disqualified. Finally, we take a closer look at the time course of the pharmacological data using a chemometric approach incorporating the uncertainty inherent to in vivo recordings, as well as the variability between CFMs to show that the drug response is significant earlier than shown by conventional statistical tests.

Accurate measurements of ambient serotonin are essential for establishing serotonin's physiological impact, and here, we present an ideal tool and analyses for this measurement. Furthermore, FSCV and FSCAV can be combined at a single sensor, providing the distinctively powerful analytical capability of measuring both phasic and ambient serotonin.

EXPERIMENTAL SECTION

Solutions

Dopamine hydrochloride, serotonin hydrochloride, 5-hydroxyindole acetic acid, ascorbic acid, 3,4-dihydroxyphenylacetic acid, uric acid, norepinephrine hydrochloride, hydrogen peroxide, adenosine, histamine hydrochloride, pargyline hydrochloride, desipramine hydrochloride, and GBR 12909 were purchased from Sigma-Aldrich (St. Louis, MO). Liquion (LQ-1105, 5% by weight Nafion) was purchased from Ion Power Solutions (New Castle, DE). Buffer solution was composed of 15 mM Tris, 126 mM NaCl, 2.5 mM KCl, 25 mM NaHCO₃, 2.0 mM NaH₂PO₄, 1.2 mM Na₂SO₄, 1.2 mM CaCl₂, and 2.0 mM MgCl₂ (all purchased from Sigma-Aldrich (St. Louis, MO)).

Carbon-Fiber Microelectrodes

The carbon-fiber micro-electrodes were assembled by aspirating a single T-650 carbon fiber (7 μ m, Goodfellow, Coraopolis, PA) into cylindrical glass capillaries (internal diameter: 0.4 mm, external diameter: 0.6 mm, A-M Systems, Carlsborg, WA). The carbon filled capillaries were positioned vertically in a pipet puller (Narishige Group, Setagaya-Ku, Tokyo, Japan) to form a carbon–glass seal under gravity. The carbon fibers were then cut to approximately

150 μm in length. Nafion solution (Liquion-1105-MeOH, Ion Power, DE) was electrodeposited on the exposed carbon fibers as previously described.⁵ The microelectrodes were dried at 70 °C for 10 min.

Data Collection

FSCV and FSCAV were performed using software (WCCV 3.05) and instrumentation developed by Knowmad Technologies LLC (Tucson, AZ). FSCAV was performed using a CMOS precision analog switch, ADG419 (Analog Devices) to control the application of the computer-generated waveform to the electrode. The logic was controlled programmatically and either a series of ramps (0.2–1.0 V to –0.1–0.2 V, scan rate = 1000 V/s) was applied every 10 ms (100 Hz), or a constant potential (0.2 V) was applied to the electrode for a specified period (10 s) (controlled adsorption period).

Flow Injection Analysis

FSCV in vitro analyses were performed using flow injection analysis (FIA). CFMs were placed into a flangeless short 1/8 nut (PEEK P-335, IDEX, Middleboro, MA) with 2 mm of the tip exposed from the nut. The microelectrode-containing nut was fastened into a modified HPLC union (Elbow PEEK 3432, IDEX, Middleboro, MA). The other end of the elbow union was secured into the out-flowing stream of the FIA buffer. Two holes were drilled into the union to incorporate a reference electrode and a “waste” flow stream. The flow was maintained using a syringe infusion pump (kd Scientific, model KDS-410, Holliston, MA) at a rate of 2 mL min^{–1}. Serotonin was introduced using a rectangular pulse into the flow stream for 10 s through a six-port HPLC loop injector (Rheodyne model 7010 valve, VICI, Houston, TX).

Data Analysis

FSCV and FSCAV signals were processed using software written in-house using LabVIEW 2009. The processing includes filtering, smoothing, and signal deconvolution. For FSCAV, the cyclic voltammogram (CV) of the third scan (after controlled adsorption period) was extracted and the peak that occurred between approximately between 0.4 V to 0.85 V was integrated. The resulting charge value in pC was plotted vs serotonin concentration to create calibration curves which were then utilized to report in vivo values.

Statistical Analysis

On the basis of the calibration data, linear models relating charge with both concentration and different electrodes were developed. These linear models incorporated interactions between the concentration and the electrode to accommodate for the inherently varying responses between electrodes.^{22,23} These models allow different intercept and slope in the linear relationship between charge and concentration for each electrode. The fitting was performed using linear model via the *lm* command in the R programming package. The results show significant differences in the intercept and slope for each electrode (Figure S-1). Using these fitted linear models, and given charge measurements collected in vivo at successive time points, estimates of the concentration levels at each time point were obtained. This was done by inverting the fitted linear relationship between concentration

and charge, and a weighted pooling of the concentration estimates from each of the electrodes was performed to obtain an overall concentration level estimate at each time point. The weights are based on the inverse of the estimated variance of the concentration estimates. Point-wise confidence intervals (CI) were constructed by fitting the functional model to the time and concentration values. These 95% point-wise confidence intervals were constructed when the functional model was fitted to the pairs of time and concentration values using the *predict.lm* command in the R package.

Exclusion Criteria

FSCV was performed before FSCAV collection in vivo to verify the presence of serotonin. CVs collected during an evoked response in mice were compared with previously well-established signals.⁵ Mice in which the CVs did not match the characteristics of a serotonin CV were excluded from this study. Furthermore, mice that died before the end of the collection time were excluded. All other mice were included in this study.

Animal Surgeries

Six-week-old male C57BL/6J mice, 20–25 g, were purchased from Jackson Laboratories (Bar Harbor, ME). The mice were housed in 12-h light/dark cycles and were offered food and water ad libitum. Animal care and procedures were in agreement with the Guide for the Care and Use of Laboratory Animals, accepted by the Institutional Animal Care and Use Committees (IACUC) of the University of South Carolina. After an intraperitoneal (i.p.) injection of the anesthetic urethane, (25% dissolved in 0.9% NaCl solution, Hospira, Lake Forest, IL) at a volume of 7 μ L per 1 g mouse weight, stereotaxic surgeries (David Kopf Instruments, Tujunga, CA) were performed. A heating pad (Braintree Scientific) was used to maintain ideal mouse body temperature of 37 °C. Bregma was used as a reference for stereotaxic coordinates of Medial Forebrain Bundle (MFB) [AP, −1.58; ML, +1.0; DV, −4.8 to −5.0] and CA2 [AP, −2.9; ML, +3.35; DV, −2.5 to −3.0] from Franklin and Paxinos (2008). To access the CA2 and MFB, holes were drilled in accordance to the stereotaxic coordinates. A stainless steel electrode (diameter = 0.2 mm; Plastics One, Roanoke, VA) was implanted into the MFB for stimulation. The nafion coated CFM was then lowered into the CA2. A silver wire (diameter: 0.010 in A-M Systems, Sequim, WA), electroplated with chloride by immersion of the wire in hydrochloric acid (0.1 M, 4 V vs tungsten), was implanted into the opposite hemisphere of the CA2 electrode placement. A 60 Hz biphasic 350 μ A, 120 pulse stimulation, 2 ms per phase was employed through linear constant current stimulus isolator (NL800A Neurolog; Digitimer Ltd.). All drugs were administered i.p.; Pargyline at a dose of 75 mg kg^{−1} and GBR 12909 at a dose of 15 mg kg^{−1}, both dissolved in 90% saline and injected at a volume of 0.1 mL 20 g^{−1}.

RESULTS AND DISCUSSION

Serotonin FSCAV

A robust analytical measurement of ambient serotonin would lend nuance to our understanding of this complex neurotransmitter. Researchers have conventionally relied on microdialysis for basal measurements; however, it is greatly desirable to us to measure this ambient concentration at CFMs. Because FSCV relies on background-subtraction to remove

a large charging current, FSCV could not, until recently, report basal neurotransmitter concentrations. We recently described a novel modification to the FSCV technique that allowed us to measure ambient in vivo DA levels, which we coined FSCAV.¹⁶ FSCAV is similar in concept to adsorptive stripping voltammetry whereby analytes adsorb onto the electrode surface for a controlled period of time before electrochemical characterization. We utilize the terminology “ambient” to denote a measurement made at a temporal resolution (20 s) that is neither on the same scale as FSCV (milliseconds) or microdialysis (minutes). The temporal scale of FSCV allows it to measure phasic changes whereas microdialysis can measure tonic or basal changes in the brain. Conversely, FSCAV is an average of both. Here, FSCAV was applied to serotonin analysis.

Electrochemical measurements of serotonin are fundamentally challenging because of serotonin's and serotonin metabolites' detrimental effects on the electrode surface. In 1995, Jackson et al. developed a solution for electrode fouling for serotonin measurements by developing a waveform, at a very high scan rate, to “outrun” fouling reactions.⁴ The “Jackson waveform” was later combined with an electrodeposited Nafion coating on the CFM for in vivo serotonin FSCV.⁵ We therefore applied the Jackson waveform (0.2 to 1.0 to -0.1 to 0.2 V, 1000 V s⁻¹) to a Nafion coated CFM for in vivo serotonin FSCAV (100 Hz) with a 10 s controlled adsorption period.

Figure 1A(i) shows a color plot of 100 nM serotonin collected in vitro with FSCV using the Jackson Waveform at 10 Hz. Figure 1A(ii) shows a color plot of 100 nM serotonin in vitro with FSCAV using the same waveform at 100 Hz. The black area in Figure 1A(ii) is the controlled adsorption period, and the CV taken at the third scan (denoted by star) after waveform reapplication is shown in Figure 1B. Importantly, this CV contains peaks characteristic of serotonin's redox potentials.^{4,5} This CV is superimposed onto a CV taken from an FSCV color plot (denoted by star). The orange lines illustrate the integration limits used for FSCAV analysis (see Experimental Section). The CVs show good agreement, with the exception of a slight potential shift in the FSCAV signal which we attribute to the higher waveform application frequency (100 Hz vs 10 Hz for FSCV).²⁴

Serotonin FSCAV is Stable, Selective, and Sensitive

We characterized this method's stability, selectivity, and sensitivity in vitro. First, in Figure 2 FSCAV files were collected in a 100 nM serotonin solution every 30 s over 120 min. An ANOVA test performed under linear models on this response shows that the measurement is highly reproducible ($p = 0.95$), affirming the method's stability in vitro.

Second, we identified 9 electroactive species in the hippocampus that could potentially interfere with the FSCAV signal.^{18,25–32} Figure 3 shows CVs collected in vitro from these different species at concentrations that mimic a range of reported or predicted physiological values.^{4,5,16,24,29,33–39}

We applied the integration limits for serotonin analysis (+0.4 to +0.85 V) and analyzed these CVs. Histamine (HA) (1 μ M), adenosine (1 μ M), DOPAC (2 μ M), norepinephrine (NE) (1 μ M), uric acid (UA) (1 μ M), DA (100 nM), ascorbic acid (AA) (200 μ M), and hydrogen peroxide (H₂O₂) (1 mM) showed no significant features within the serotonin integration

limits ($n = 4$). 5-HIAA ($10 \mu\text{M}$)⁵ could prove problematic due to the presence of a peak within the integral limits.

We show that 5-HIAA interference is unlikely in Figure 4. In this figure, charge is plotted against concentration for serotonin (orange). Here, the linear portion of the serotonin calibration is shown with orange markers with the following linear regression:

$$y=0.0207(\pm 0.0005)x+1.51(\pm 0.14), \quad R^2=0.997 \quad (1)$$

The serotonin plot shows linearity up to 600 nM, with a sensitivity of $0.021 \pm 0.0005 \text{ pC nM}^{-1}$ ($n = 4 \pm \text{SEM}$), and a limit of quantification of 5 nM. When both serotonin and 5-HIAA are present in solution, we postulate that there is a competition for adsorption sites on the carbon fiber surface. The rationale here is that the analyte with higher adsorption equilibrium constant (K_{ads}) on the CFM will exhibit a more favorable thermodynamic adsorption profile and will thus outcompete the other.⁴⁰ A Langmuir monolayer adsorption isotherm model was used with FSCAV data to calculate K_{ads} for serotonin and 5-HIAA. K_{ads} for serotonin and 5-HIAA were 9.57×10^{10} and 7.02×10^8 , respectively. The much higher K_{ads} for serotonin adsorption onto CFMs means that 5-HIAA added to serotonin does not affect the signal as shown in the inset in Figure 4. The green stars signify a separate data set where approximately 100× more concentrated 5-HIAA was added to the serotonin solution and the blue markers show the signal. The close agreement of the blue and orange markers make it clear that 5-HIAA, at physiological concentrations (typically $10 \mu\text{M}$),⁴¹ would not impact the signal. In addition to favorable adsorption, the much improved sensitivity for serotonin vs 5-HIAA can be credited to Nafion on the CFM.⁵

In Vivo Serotonin FSCAV

To apply FSCAV to in vivo serotonin measurements, we first employed a retrograde stimulation of the medial forebrain bundle (MFB) and confirmed electrically stimulated serotonin release in the CA2 region of the mouse hippocampus (example of evoked release can be seen in Figures 6 and 7). Subsequently FSCAV was performed at the same electrode. Figure 5A shows the in vivo FSCAV color plot (i) adjacent to a color plot of 100 nM serotonin in vitro (ii).

The close agreement of the CVs shown in Figure 5B is strong evidence that this signal is serotonin. We took a chemometric approach to report the concentration in vivo with uncertainty that incorporated not only in vivo variability but also the variability of individual electrodes. These variations occur mainly due to the nonuniformity between carbon fiber surfaces that arise as a result of the fabrication process. A linear functional model was developed using the calibration data of the different electrodes used in the in vivo experiments. In 15 mice, the weighted average extracellular serotonin level was $64.9 \pm 2.3 \text{ nM}$ ($n = 15$ mice, weighted average $\pm$ standard error) (see inset in Figure 5B).

Previous reports of ambient serotonin with microdialysis in different brain regions have estimated extracellular serotonin in low nanomolar to 10s of nanomolar.^{9–13} Our value is slightly above this range. Our method is performed on a fundamentally different spatial

scale. For example, commercial microdialysis probes typically have a diameter of 200 μm and are 2 mm in length, whereas CFMs are 7 μm in diameter and 150 μm in length. The tissue volume impacted by a CFM is orders of magnitude smaller than that of a typical microdialysis probe¹⁵ and because we optimize the electrode's placement based on stimulated serotonin release, the electrode is in a "hot spot" (area of high serotonin activity), accounting for slightly higher levels.

Above, we assessed FSCAV's selectivity in vitro, however, the in vivo matrix is far more complicated than can be reproduced on the bench. It is therefore critical to verify the signal pharmacologically in vivo. For this task, we employed pargyline (75 mg kg⁻¹), an irreversible monoamine oxidase B (MAO-B) inhibitor. By inhibiting MAO-B, and hence the metabolism of serotonin in the brain, an increase in serotonin and a decrease in 5-HIAA is expected.^{42,43} Figure 6 shows experiments that test the effects of pargyline on the FSCAV signal. First, FSCV was used to optimize the position of the CFM by evoking serotonin release (a representative example color plot is shown inset on the top left). Individual animal FSCAV responses (faint blue markers) and the averaged response (dark blue dots) 60 min before and 60 min after an i.p. injection of pargyline are shown on the central trace. Using conventional statistical analysis, pargyline administration caused a significant increase in the FSCAV signal at 29 min and thereafter (two way repeated measures ANOVA: $p < 0.0001$, $n = 5$ mice $\pm$ SEM with Dunnett's multiple comparison post hoc, $p < 0.01$, $n = 5$ mice $\pm$ SEM). The effects of pargyline were verified with FSCV following FSCAV data collection (inset top right color plot is a representative color plot and [serotonin] vs time traces (α = predrug and β = postdrug)), where pargyline increased evoked serotonin amplitude and reduced the rate of reuptake as previously seen in rats.⁶ This experiment eliminates the possibility of 5-HIAA interference and would verify our signal as serotonin, save for one final concern; that DA and NE are also substrates for MAO-B^{44,45} and are present at appreciable levels in the CA2 region of the hippocampus.^{17,20,21}

Very little sensitivity was established in vitro for DA or NE. To further verify no interference from DA we administered GBR 12909, a potent DA transporter inhibitor, to a separate set of mice. We have previously shown that GBR 12909 causes an increase in ambient DA,¹⁶ but not in evoked serotonin levels.⁵ The faint, red markers in Figure 6 show individual FSCAV animal responses to 15 mg kg⁻¹ GBR 12909, while the dark red dots show the averaged responses 60 min before and after i.p. administration ($n = 5$ mice $\pm$ SEM). As above, FSCV was used to assess the effects of this manipulation on the evoked serotonin response. The lack of an increase in the FSCAV signal (two way repeated measures ANOVA, $p > 0.05$, $n = 5$ mice $\pm$ SEM with Dunnett's multiple comparison post hoc, $p > 0.05$, $n = 5$ mice $\pm$ SEM) and FSCV signal allows us to exclude DA as interference.

To eliminate the possibility of interference from NE, we administered desipramine (15 mg kg⁻¹) to a separate set of mice. Desipramine is a norepinephrine transporter (NET) inhibitor that selectively blocks NETs but has negligible effect on DA or 5HT transporters.^{46,47} The faint green, markers in Figure 7 represent the individual FSCAV responses, while the dark green dots represent the averaged response 60 min before and after i.p. drug administration ($n = 5$ mice $\pm$ SEM). Injection is immediately before first data point at 0 min (yellow bar). FSCV color plots and CVs taken before and after FSCAV data collection from a

representative experiment are inset. There was no change in the FSCAV signal (two way repeated measures ANOVA, $p > 0.05$, $n = 5$ mice $\pm$ SEM with Dunnett's multiple comparison post hoc, $p > 0.05$, $n = 5$ mice $\pm$ SEM). There was no increase in the release amplitude as measured with FSCV, however there was a dramatic dip below baseline after stimulation. We previously showed that dips such as this were mediated by prolonged autoreceptor activation⁷ and because desipramine has agonist activity at the 5H1B receptor,^{48,49} it is likely we are observing a potentiation of the autoreceptor effect. This experiment allows us to exclude norepinephrine as a possible interference.

Conventional statistical tests (e.g. two way repeated measures ANOVA with Dunnett post hoc test) show that the serotonin levels increase significantly 29 min after pargyline administration. However, visually it is seen that the serotonin levels begin to rise much earlier than that. To address this, we expanded the fitted linear model to encompass the concentration values prior to and post pargyline, GBR 12909, and desipramine administration. The equations of the model were as follows

serotonin/pargyline

$$C(t) = 65.217 + 0.0041 \times t + 0.5268 \times [\max(0, 62.6 - t)] - 0.0042 \times [\max(0, 62.6 - t)]^2 \quad (2)$$

serotonin/GBR12909

$$C(t) = 58.82 + 0.0055 \times t - 0.000088 \times t^2 \quad (3)$$

serotonin/desipramine

$$C(t) = 71.54 + 0.0013 \times t - 0.000004 \times t^2 \quad (4)$$

where $C(t)$ is change in concentration with time, t is time and $\max(a, b)$ is the larger value between a and b . The fitted model (blue line) over the averaged serotonin data (black dots) with pargyline, GBR 12909, and desipramine administration is seen in Figure 8A–C, respectively.

For pargyline, the functional continuous model consisted of a linear part over the time portion where no drugs were administered plus a time lag Delta (0 to 60 + Delta), and is parabolic over the time interval from 60 + Delta to 120 (Figure 8A). Using this model, the estimate for this time lag Delta or simply the time point where the drug causes a change in the slope is 2.60 min. This estimate is obtained by maximizing the coefficient of determination (R^2) with respect to the possible values of Delta. Note that the final fitted model has a high R^2 equal to about 95%, indicating an excellent fit of the linear-parabolic model for relating concentration to time for this serotonin study.

For the GBR12909 and desipramine, the model showed no effect of time on serotonin concentration and there was basically no change over the whole period of study. The plot of this fitted model is presented in Figure 8B and 8C, respectively, which is almost flat, together with the estimated concentration levels (the solid circles) at each of the time points, and the 95% point-wise confidence intervals.

It is important to note that using repeated measures ANOVA with Dunnett test, the only information that was available is the time point at which pargyline caused a change that was statistically significant ($p < 0.05$, 29 min). On the other hand, through employing a statistical model that was built to take into account electrode variability, we were able to determine the point at which pargyline changed the ambient serotonin concentrations as soon as 2.60 min. This may be a more accurate reflection of the pharmacological profile of this agent.

The combination of electrochemical and pharmacological characterizations performed in vivo and in vitro allows us to confidently assert that FSCAV is able to selectively measure ambient serotonin in vivo. The synergy with a chemometric approach introduces a new wealth of information that allows for more accurate electrode calibrations and a more comprehensive understanding of the time course of in vivo data.

CONCLUSIONS

Imbalances in serotonin neurochemistry are important to study in the context of neuropsychiatric disorders. While FSCV can provide real-time chemical information, the method reports only phasic changes. Ambient serotonin levels are critical to establishing the fundamental extracellular mechanisms that control serotonin. Here, we reported FSCAV for ambient serotonin measurements. We performed a characterization of the FSCAV waveform for sensitive and selective serotonin measurements. In vivo, we utilized the waveform to report a basal serotonin level in mouse CA2 as 64.9 ± 2.3 nM ($n = 15$ mice, weighted average $\pm$ standard error). We pharmacologically verified the in vivo signal against perceived interferences. Finally, we developed a statistical model to further analyze the FSCAV readings and report the uncertainty caused by measuring in vivo using different CFMs. Serotonin FSCAV yields information about serotonin's basal behavior in vivo and, when coupled with FSCV at a single CFM, will provide a deeper chemical insight into serotonin's mechanisms in the brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. (A) Representative FSCV (i) and FSCAV (ii) color plots of 100 nM serotonin in vitro. (B) Cyclic voltammograms extracted from the vertical dashed lines in A(i) and A(ii) after normalization (current/maximum current). Vertical orange dashed lines represent integration limits.

Figure 2. Repeated FSCAV measurements over 120 min in 100 nM serotonin ($n = 4$ electrodes $\pm$ SEM).

Figure 3.

In vitro CVs for HA (1 μM), adenosine (1 μM), DOPAC (2 μM), NE (1 μM), UA (1 μM), DA (100 nM), AA (200 μM), H_2O_2 (1 mM), and 5-HIAA (10 μM). Vertical dashed lines represent integration limits utilized for serotonin analysis.

Figure 4.

Serotonin selectivity curve ($n = 4$ electrodes $\pm$ SEM). The inset shows linear serotonin range (orange markers), the green stars represent the addition of 5-HIAA to serotonin. All blue markers represent serotonin/5-HIAA mixture with 5-HIAA being a 100 times the serotonin concentration. All inset calibrations are $n = 4$ electrodes $\pm$ SEM.

Figure 5. (A) Representative FSCAV color plots of serotonin in vivo (i) and in vitro (ii). (B) CVs extracted from the 3rd scan indicated by vertical dashed lines in A(i) and A(ii). The inset shows ambient serotonin measurements in CA2 region of mouse hippocampus. Gray markers represent individual mice and orange marker represents weighted averaged response ($n = 15$ mice $\pm$ standard error).

Figure 6.

Faint blue markers represent individual mouse responses to i.p. Pargyline (75 mg kg^{-1}) and faint red markers represent individual mice responses to i.p. GBR 12909 (15 mg kg^{-1}). Files were collected 60 min before and after drug administration. Dark blue dots represent averaged Pargyline response ($n = 5 \text{ mice} \pm \text{SEM}$) and dark red dots represent averaged GBR 12909 response ($n = 5 \text{ mice} \pm \text{SEM}$). Yellow bar at 0 min is injection time. Representative FSCV color plots and CVs before and after FSCAV file collection are inset (top, pargyline; bottom, GBR 12909, α = predrug and β = postdrug). White bars at bottom of color plot denotes stimulation (2 s). Inset center shows [serotonin] vs time traces taken from color plots. Red bars below [serotonin] vs time is the stimulation. (Asterisks above solid blue markers indicate post hoc test: * $p < 0.05$ and **** $p < 0.0001$.)

Figure 7.

Faint green markers represent individual mice responses and dark green dots represent averaged response to i.p. Desipramine (15 mg kg⁻¹) ($n = 5$ mice $\pm$ SEM). Files were collected 60 min before and after drug administration. Yellow bar at 0 min is injection time. Representative FSCV color plots and CVs before and after FSCAV file collection are inset (α = predrug and β = postdrug). White bars at bottom of color plot denotes time of stimulation (2 s). Inset center are representative [serotonin] vs time traces of evoked serotonin response before (black) and after (green) drug administration. Red bars below [serotonin] versus time is stimulation period (2 s).

Figure 8.

Files were collected 60 min before and after (A) pargyline, (B) GBR12909, and (C) desipramine administration. Circles represent averaged serotonin response ($n = 5$ mice $\pm 95\%$ CI). Vertical gray lines represent 95% confidence intervals, and the blue line is the fitted model. Red vertical line in A represent point of change after drug administration, that is, 2.60 min.