Supplementary Figure 1. Microenvironment surrounding the chronic microsensor.

10 × composite images of 30-µm striatal sections were taken from rats implanted with a microsensor for a period of 10 to 16 weeks (n = 5). Striatal sections taken 1 mm anterior to the slice containing the carbon fiber were stained as a control. Astrocytes were marked with a GFAP antibody (red), microglia were labeled with the Iba1 antibody (green), and TH was used as a marker of dopaminergic synapse density (blue). While a small amount of astrogliosis is observed dorsal of the anterior commissure in the first composite image, a large astrocytic response was not observed in any of the other samples. Along the silica shaft of image 2 and 5 some activated microglia can be observed (by their condensed shape), but in all cases no glial cap is observed around the microsensor. The intensity of TH was measured using 300 µm line scans (150 µm radiating from the center of the silica). The middle white line in all images (100 µm below the epoxy) marks the area where TH was measured for the carbon fiber, the top white line marks the area measured for the silica shaft (500 µm from the epoxy), and the bottom white line marks the area measured for control TH expression. The five samples show significant decreases in the z-score of for TH intensity at the level of the silica shaft but no significant loss was observed around the carbon fiber, compared to control.
Supplementary Figure 2. Electrically evoked dopamine release measured with acute and chronic microsensor preparations.

Traces depict electrically evoked dopamine release (stimulation at time 0) measured by chronic microsensors (black line n = 5) and by conventional glass insulated acutely implanted electrodes (blue line n = 5). Stimulating electrodes were lowered into the ventral tegmental area where electrical pulses (60 Hz, 24 pulses at 120 μA) elicited dopamine release at the recording location in the nucleus accumbens. Data are presented as mean ± s.e.m. The inset depicts the corresponding normalized background-subtracted cyclic voltammograms.
Supplementary Figure 3. Comparison of cyclic voltammograms obtained *in vitro* and from multiple *in vivo* preparations.

Average normalized background-subtracted cyclic voltammograms from multiple experimental preparations (left panel; \( n = 5 \) for each group). Statistical comparison of the average CVs obtained from each preparation revealed significant correlations between all groups (right panel; all \( r^2 \geq 0.75 \)).
Supplementary Figure 4. Pharmacological validation of in vivo voltammetric signals.

Comparison between the effect of artificial cerebrospinal fluid (ACSF; blue bar) and baclofen (50 ng; red bar) microinjections into the ventral tegmental area (VTA) on the voltammetric signal elicited by reward delivery at chronically implanted electrodes (two months after implantation). The schematic drawing depicts the saggital view of a rat brain illustrating injection sites in the VTA. Baclofen significantly reduced behaviorally evoked dopamine release in comparison to ACSF (two tailed paired t-test, $t = 3.062$, $P < 0.05$, $n = 5$; left panel). Data are presented as mean ± s.e.m., normalized to pre-injection dopamine release. Representative traces of the voltammetric signal in the nucleus accumbens core from a single animal in response to reward delivery before (black) and after local microinjection of ACSF (blue) or baclofen (red) into the VTA (right panel). Each tick mark denotes one second.
Supplementary Figure 5. Multi-site, simultaneous recording.

(a) Simultaneous recording of dopamine release in the nucleus accumbens elicited by reward presentation. The left panel shows an example of concurrent dopamine release bilaterally in the NAc core. The right panel shows an example of concurrent dopamine release in the NAc core and contralateral NAc shell. The insets depict the corresponding normalized background-subtracted cyclic voltammograms. Each tick mark denotes 1 second. (b) A stimulating electrode was lowered into the medial forebrain bundle where electrical pulses (60 Hz, 120 pulses at 300 μA) elicited dopamine release that was concurrently recorded in four areas of the striatum ranging from the dorsolateral striatum to the NAc. The experimental set-up is schematized and recording locations are indicated by the colored circles (left panel). Simultaneous dopamine release following electrical stimulation is shown for the four striatal locations (colored coded to indicate the recording locations depicted in the left panel) with corresponding normalized background-subtracted cyclic voltammograms in the inset (right panel). Each tick mark denotes 1 second.
Supplementary Table 1. Failure mode of the chronically implanted electrodes presented in Figure 2a.

| Rat ID | Reason for attrition (implantation days to last successful recording*) |
|--------|-----------------------------------------------------------------------|
| 1      | Separation of cranioplastic implant (79)                              |
| 2      | Infection around cranioplastic implant (103)                          |
| 3      | Increased electrical noise (88)                                      |
| 4      | Saturated background signal after rat chewed headstage cable (133)   |
| 5      | Small signal to stimulus and increased electrical drift (162)        |
| 6      | Infection around cranioplastic implant (97)                          |
| 7      | Infection around cranioplastic implant (0¹)                           |
| 8      | Consistent artifact following stimulus (97)                          |
| 9      | Infection around cranioplastic implant (86)                          |
| 10     | Infection around cranioplastic implant (69)                          |
| 11     | Intermittent electrical connection (0¹)                               |
| 12     | Small signal to stimulus (51)                                        |
| 13     | Intermittent electrical connection (48)                               |
| 14     | Consistent artifact following stimulus (72)                          |
| 15     | Saturated background signal (0¹)                                     |
| 16     | Consistent artifact following stimulus (126)                         |
| 17     | Consistent artifact following stimulus (99)                          |
| 18     | Separation of cranioplastic implant (126)                            |
| 19     | Intermittent electrical connection (68)                               |
| 20     | Intermittent electrical connection (0¹)                               |

* Recording sessions were classified as successful if the electrochemical signal elicited by reward delivery met criterion for dopamine (see Methods: Data Analysis)

† Problems arose prior to or during first test that prevented subsequent recording
Supplementary Table 2. Cross comparison of cyclic voltammograms obtained from a single animal over the course of four months *in vivo* and a dopamine standard *in vitro*.

|               | Stim, month 1 | Reward, month 1 | Reward, month 2 | Reward, month 3 | In vitro |
|---------------|---------------|-----------------|-----------------|-----------------|----------|
| Stim, month 1 | 0.97          | 0.96            | 0.97            | 0.92            | 0.84     |
| Stim, month 2 | 0.95          | 0.95            | 0.95            | 0.85            |          |
| Reward, month 1 | 0.93      | 0.86            | 0.92            |                 |          |
| Reward, month 2 | 0.91      | 0.87            |                 |                 |          |
| Reward, month 3 |           |                 |                 | 0.81            |          |
Supplementary Note

Characterization of in vivo Voltammetric Signals

In order for the current advances in voltammetric microsensors to be experimentally useful, the microsensor and applied voltammetric technique must have the capacity to resolve and identify extracellular changes in dopamine, the primary analyte of interest. It is therefore necessary that the in vivo signals detected by the microsensor be adequately characterized. Four broad criteria have been proposed for the characterization of in vivo voltammetric signals: electrochemical, anatomical/physiological, pharmacological and independent chemical verification\(^1\)\(^3\). Although both FSCV and the carbon fiber used in these microsensors have already been well characterized for the detection of dopamine\(^1\)\(^4\)\(^5\), we apply these criteria in order to evaluate our chronic microsensor and determine whether its properties are altered during chronic implantation.

The electrochemical-verification criterion can be satisfied by correlating cyclic voltammograms obtained from behaviorally evoked signals to a reference standard generated by electrical stimulation of a known source of dopamine input (VTA, SNc, or medial forebrain bundle). In FSCV, voltage is applied in a triangular waveform generating an output current profile, which when presented as a current-voltage plot (the CV) provides a chemical “signature” for the compound of interest. Correlation coefficients between CVs resulting from stimulations of the VTA, after reward presentation and exogenous dopamine standards in vitro are shown in Supplementary Figure 3 for the chronically implanted microsensor and, for comparison, acutely implanted electrodes. Behaviorally evoked release was highly correlated \((r^2 \geq 0.75)\) with all of the dopamine standards tested.

The second criterion, anatomical and physiological verification, can be satisfied by showing that the neurochemical of interest is present at the recording site and demonstrating that the tissue is capable of releasing the neurochemical with the observed kinetics. All electrode placements were histologically confirmed to be in the ventral or dorsal extents of the striatum, structures previously shown by tissue measurements to be high in dopamine content\(^6\). In addition, immunohistological assessment of the microenvironment being sampled by the microsensor indicates that TH, the biosynthetic enzyme for dopamine, is present in the surrounding tissue (Fig. 1b and Supplementary Fig. 1). These brain regions have been shown to support high rates of dopamine release as indicated using electrical stimulation of afferent pathways shown previously\(^7\) and in Figure 1c. In addition, the dynamics and timing of data shown in Figure 2c from the chronically implanted microsensors are consistent with electrophysiological studies examining the firing pattern of dopamine neurons in similar behavioral paradigms\(^8\)\(^9\). Specifically when rewards are preceded by sensory stimuli, dopamine release is initially observed at the time of the reward, but once the stimulus becomes a predictor of reward, dopamine is released at the time of the stimulus and not the reward. Together, these findings indicate that the microsensor is responsive on a physiologically relevant timescale.

Pharmacological verification involves the assessment of an evoked signal after the application of an agent known to disrupt the source of the signal. To meet this third criterion, the behaviorally evoked signal at the chronically implanted microsensor was measured before and after an infusion of the GABA\(_B\) receptor agonist baclofen (50 ng) into the VTA. Previous work has demonstrated that the majority of dopamine neurons in the VTA express GABA\(_B\) receptors, and that activation of GABA\(_B\) functionally inactivates dopamine neurons via hyperpolarization\(^10\)\(^11\). Consistent with these findings, food-evoked electrochemical signals were significantly attenuated by intra-VTA baclofen (to 26.0 ± 24.7 % of baseline; two tailed paired t-test, \(t = 3.062, P < 0.05, n = 5\)) but were not affected following vehicle infusion (101.5 ± 21.6 %; \(n = 5\)) in the same animals (Supplementary Figure 4), again indicating that the signal is dopamine.

The final criterion for the characterization of an in vivo signal of interest is independent chemical verification which provides an opportunity to test whether the chronically implanted microsensor can perform at the level of existing methodologies. Rapid changes in dopamine transmission after the presentation of salient stimuli, including reward presentation and reward-predictive cues, have been previously demonstrated with FSCV at acutely implanted electrodes\(^12\)\(^13\). Therefore it is important to verify that comparable chemical signals are observed in the chronically-implanted environment. As demonstrated in Figure 2c, phasic signals during reward presentation and in response to reward predictive cues recorded day-to-day at the chronically implanted electrode are extremely consistent with the “snapshots” provided by acutely implanted electrodes at
specific time points. Thus, well-characterized recordings from the acutely implanted environment provide independent chemical verification of the data presented with the current approach.

We have presented a comprehensive characterization of the chronically implanted microsensor for the detection of a behaviorally relevant stimulus (food-pellet presentation) using standard signal-identification criteria\(^2,3\) based upon electrochemical, anatomical/physiological, pharmacological and independent verification. Collectively, the findings demonstrate the capability of these chronic microsensors to make chemically selective, subsecond recordings in behaving animals.

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