Supplemental Digital Content for

A pain-induced tonic hypodopaminergic state augments phasic dopamine release in the nucleus accumbens

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This PDF file includes:

Supplementary Information Text

Electrode Fabrication

Cylindrical carbon-fiber microelectrodes (CFMEs) were fabricated from AS-4 7 μm-diameter carbon fibers (Hexcel Corporation, Stamford, CT, USA). Single carbon fibers were aspirated into glass capillaries and pulled with a vertical micropipette puller (Narashige, Tokyo, Japan). These nascent electrodes were then cut to 60-80 μm in length. Silver wires (Kauffman Engineering, Inc., Cornelius, OR, USA) were coated with alcohol-based graphite conductive adhesive (Alfa Aesar, Ward Hill, MA, USA) and inserted into the back end of the nascent electrodes for electrical connectivity. Epoxy adhesive (Henkel Corp., Rocky Hill, CT, USA) was applied to the electrode and allowed to dry overnight to secure the wire. Electrodes were then coated with a 3,4-ethylenedioxythiophene and Nafion (PEDOT:Nafion) polymer blend to increase dopamine sensitivity and selectivity.[2] Ag/AgCl reference electrodes used in all experiments were created by soaking 0.25 mm diameter silver wires (Alfa Aesar, Ward Hill, MA, USA) in bleach overnight.

Electrode Placement

Before experimentation, animals were anesthetized with isoflurane (induction, 5% isoflurane/L/min maintenance, 2%/L/min, Vetone, Biose, ID, USA). Holes were drilled in the skull allowing placement of a working electrode above the left NAc shell (anteroposterior (A/P) +1.7 mm, mediolateral (M/L) +0.8 mm; relative to bregma) and an Ag/AgCl reference electrode that was implanted in the contralateral hemisphere. While lateralized differences in the right versus left NAc are possible [1], conducting experiments with the working electrode in both hemispheres is not standard practice for the field. A bipolar stimulating electrode (Plastics One, Roanoke, VA) was positioned over the medial forebrain bundle (MFB, A/P -2.5 mm, M/L +1.7 mm). The working and stimulating electrodes were placed in their respective dorsal/ventral (D/V) locations by stimulating the MFB to maximize

stimulated release in the NAc (typically D/V -6.9 to -7.5 mm for the working electrode and D/V -7.0 to -7.5 mm for the stimulating electrode). Electrode placement was conducted prior to standardization of depth of anesthesia (*vide infra*).

Power analysis

To estimate the number of subjects required in these studies power analyses were conducted. Preliminary tonic FSCAVtonic data suggested an average percent decrease in response to saline treatment of 2%, while the capsaicin-induced decrease was roughly 15%. Using a two-sample two-sided online power calculator to compare means (http://powerandsamplesize.com/Calculators/Compare-2-Means/2-Sample-Equality) a sample size of 10 was determined necessary. Input values: mean A = 2, mean B = 15, sigma= 10, ratio = 1, power 0.08, alpha = 0.05.

A similar power analysis was conducted for phasic FSCVphasic experiments. Preliminary phasic data did not suggest a change in response to saline application and demonstrated a 20 % increase in evoked phasic release after capsaicin application. Using the same software as with tonic data a necessary sample size of 4 was determined. Input values: mean A = 0, mean B = 20, sigma= 10, ratio = 1, power 0.08, alpha = 0.05.

Measurement of Tonic Dopamine Levels

$FSCAV_{tonic}$ allows for absolute measurements of extracellular dopamine by employing a 10-second holding period wherein the electrode is held at -0.4 V vs. Ag/AgCl. During this time extracellular dopamine adsorbs to the CFME surface. After the holding period, a triangle waveform (-0.4 to +1.3 V vs. Ag/AgCl, 1200 V/s) is applied to the CFME causing the oxidation and reduction of adsorbed dopamine and allowing for absolute measurements. We integrate the oxidation peak after the holding period and analyze subsequent voltammetric scans.

Calculation of Hypodopaminergic State Duration

To calculate the duration of the tonic hypodopaminergic state for each animal, first the timing of the tonic dopaminergic minimum was determined. We then calculated then length of time required for tonic dopamine levels to recover by 40% of the value by which they decreased. This point in time was referred to as the tonic dopaminergic recovery time (Figure S1). The average dopaminergic recovery time after the primary application of capsaicin to the first cornea was 2 □ 0.3 minutes. In the alternate cornea, the average dopaminergic recovery time after the primary application of capsaicin was 1.9 □ 0.3 minutes. There was no statistical difference observed between the duration of the hypodopaminergic state induced by the two primary capsaicin applications (paired t-test, t9 = 0.17, p > 0.85)

Measurement of Phasic Dopamine Signals

In this procedure, we applied a triangle waveform (-0.4 to +1.3 V vs. Ag/AgCl, 400 V/s) to the CFME every 100 ms. In between scans the electrode was held at -0.4 V. In-house LabVIEW software (National Instruments, Austin, TX, USA) along with a data-acquisition board (PCIe- 6321, National Instruments, Austin, TX, USA) generated the waveform and collected the voltammetric signal. The cyclic voltammograms are used to discriminate between analytes.

Histology

After electrode lesioning, the animals were euthanized via CO_2 asphyxiation followed by cervical dislocation. Electrode locations were verified based on lesion. Lesions in the brain were made by incrementally increasing the current applied to the electrode slowly over the course of 10 minutes and animals were analyzed according to their electrode position and excluded from analysis if

outside the NAc shell or NAc core. Representative histological verification data are shown in Figure S7. MFB tract lesions were clearly visible.

Figure S1

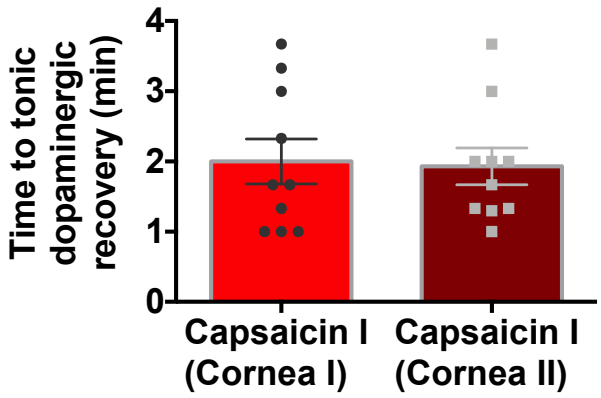

Figure S1. Average duration of tonic hypodopaminergic state. The primary application of capsaicin in the first cornea [Capsaicin I (Cornea I)] was 2.0 ± 0.3 minutes. The first application of capsaicin in the alternate cornea [Capsaicin I (Cornea II)] was 1.9 ± 0.3 minutes. No statistical difference was observed between the duration of the hypodopaminergic state induced by the two initial capsaicin applications (paired t-test, $t_9 = 0.17$, $p > 0.85$)

Figure S2

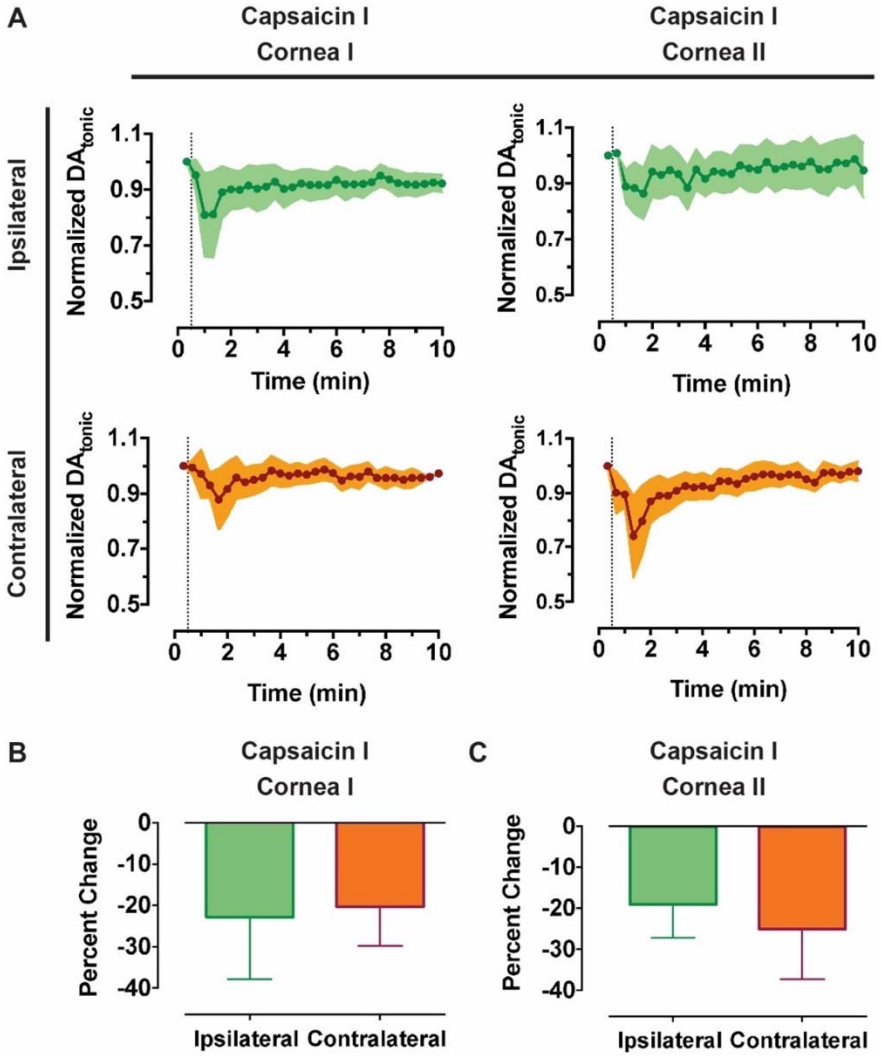

Figure S2. Lateralization of capsaicin application relative to WE does not affect tonic dopaminergic decrease. (A) (top right) Tonic dopaminergic response to ipsilateral capsaicin applied to the animals first cornea; these data correspond to a first exposure to capsaicin in the animal's first eye (n = 5 animals). (top left) Dopaminergic tone in response to ipsilateral capsaicin; these data correspond to the first exposure to capsaicin in the alternate (or secondary) eye, (n = 5 animals). (bottom right) Dopaminergic tone in response to contralateral capsaicin; these data correspond to a first (or primary) exposure to capsaicin in the first cornea (n = 5 animals). (bottom left) Tonic dopaminergic response to contralateral capsaicin; these data correspond to the first exposure to capsaicin in the animal's second (alternate) cornea (n = 5 animals). (B)

Quantification of above data, percent change upon capsaicin application was calculated for Cornea I. When the cornea ipsilateral to the WE received capsaicin first, DA_{tonic} decreased by 23 ± 15%. If the first eye of application was the contralateral eye, a decrease of 20 ± 10% was observed. No significant difference was measured. (C) Percent change upon first (or primary) capsaicin application to the alternate eye was calculated. When capsaicin application in the alternate eye was ipsilateral to the WE, DA_{tonic} decreased by 19 ± 8%. If the capsaicin application in the alternate eye was contralateral to the working electrode, DA_{tonic} decreased by 25 ± 12 %. No statistical difference was observed (unpaired t-test, $p > 0.8$). All data are represented as mean ± SEM.

Figure S3

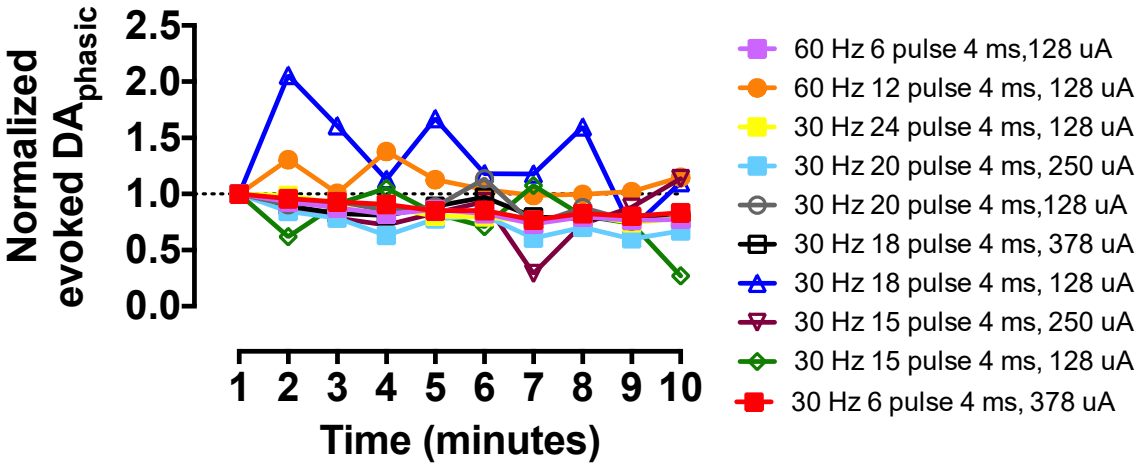

Figure S3. Determination of stimulation parameters used to evoke phasic, endogenous-like dopamine events in $FSCV_{phasic}$ sustained pain experiment. The medial forebrain bundle (MFB) was stimulated once per minute. Carbon-fiber microelectrodes detected phasic dopamine release in the NAc shell. The resultant normalized peak oxidation current (DA_{phasic}) was plotted as a function of time. The stimulation parameters chosen (red squares, 30 Hz, 6 pulses, 4 ms pulse width, 5 V, and 378 μA) had the smallest deviation from the initial stimulation (17% decrease over ten 1-minute interval stimulations) and the smallest variation throughout the ten-minute period. Ultimately, two-minute intervals were employed to further decrease the loss in stimulated release throughout the experiment.

Figure S4

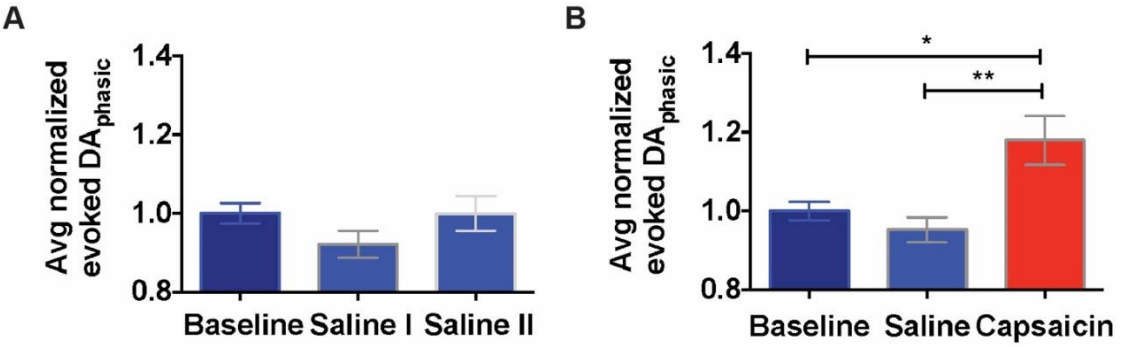

Figure S4. Capsaicin causes an increase in the average electrically evoked phasic dopamine release over ten minutes. (A) Averages of the stimulations during baseline and two consecutive saline treatment periods in a control experiment show no difference in evoked phasic dopamine release (one-way ANOVA, $F_{2,12} = 1.64$, $p > 0.20$). (B) The average of the five evoked dopamine release events after capsaicin treatment was significantly higher than the average of the five events after saline application or the baseline stimulation period (one-way ANOVA, $F_{2, 12} = 8.01$, $p < 0.01$; post-hoc Tukey's analysis revealed, $^{*}p < 0.05$ and $^{**}p < 0.01$).

Figure S5

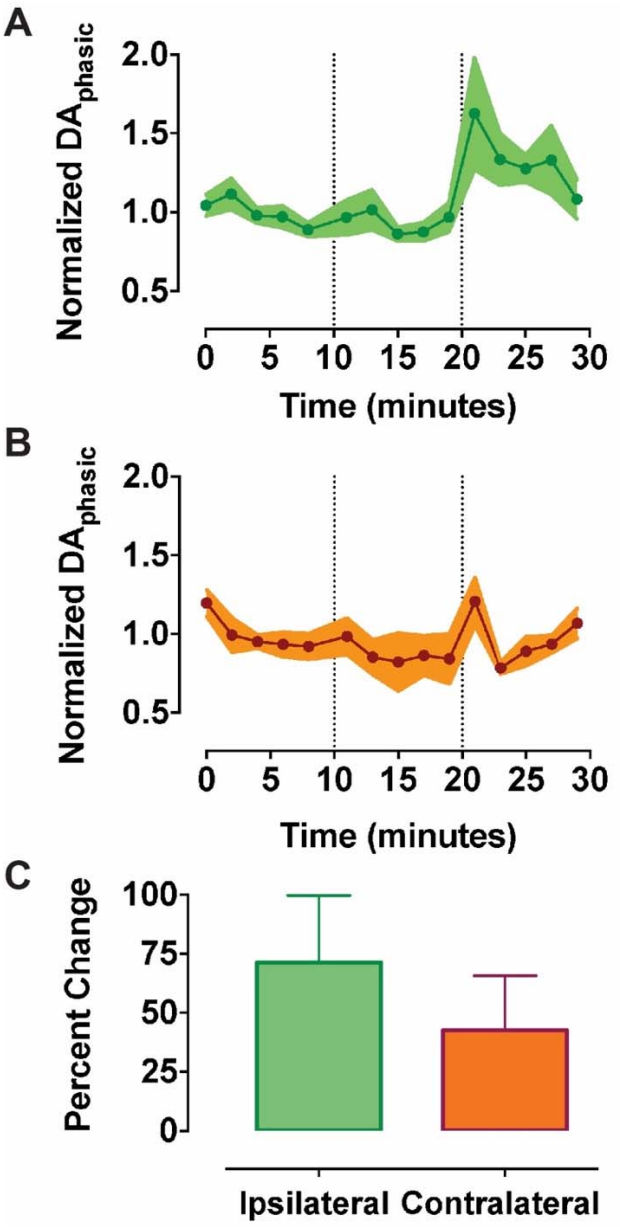

Figure S5. Phasic dopaminergic response is increased after capsaicin application in both the ipsi- and contralateral hemispheres relative to the CFME placement. (A) Phasic dopaminergic response to ipsilateral saline and capsaicin applications. First vertical dotted line represents saline application. Second dotted line represents capsaicin application. (B) Phasic dopaminergic response to contralateral saline and capsaicin treatments. (C) Percent change relative to the average of the two stimulations prior to capsaicin application in ipsilateral and contralateral

corneas. When the recording electrode was on the side ipsilateral to the hemisphere of capsaicin application a 71 ± 28%% increase in evoked phasic release was observed (n = 6 animals). When the capsaicin application was applied contralaterally to recording electrode hemisphere a 42 ± 23% (n = 6 animals) increase in evoked phasic release was measured. No significant difference was observed between ipsi- and contralateral capsaicin treatments. In all cases data are represented as mean ± SEM.

Figure S6

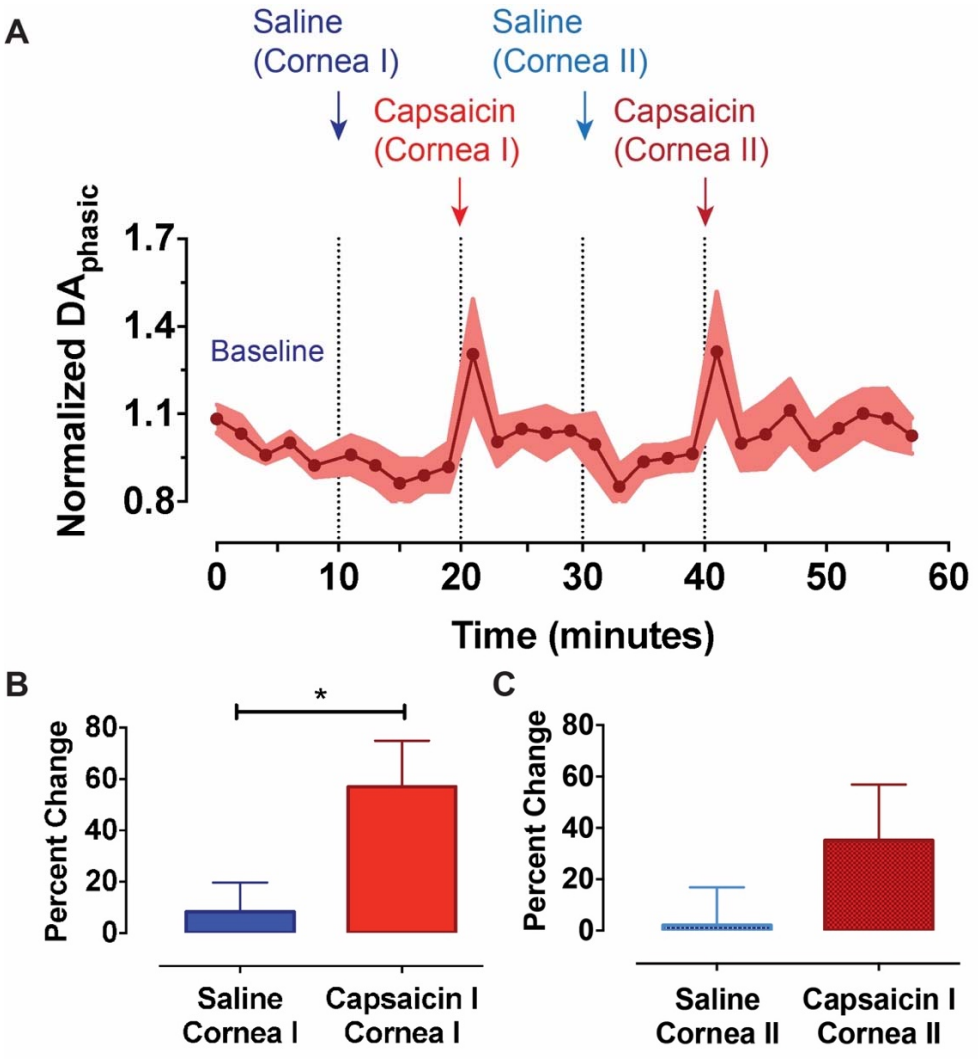

Figure S6. Sustained pain causes an increase in evoked phasic dopamine release. (A) Evoked phasic dopamine release recorded every two minutes with applications of saline and capsaicin in 10-minute intervals. The first dotted line after the Baseline stimulation period represents the first saline corneal application randomized to the ipsi- or contralateral hemisphere relative to the carbon fiber microelectrode (CFME). The second vertical line represents a capsaicin treatment in the same eye. Next, saline was applied to the alternate cornea (third vertical dotted line). Lastly, capsaicin was applied to the alternate cornea and evoked stimulations were recorded for

18 minutes thereafter. Mean ± SEM, n = 12 animals. (B) Calculated change after saline and capsaicin treatments in the first cornea. The average of the two points proceeding the application was compared to the single point directly following the treatment. The saline application in the first cornea caused an 8.3 ± 11.4% increase in evoked phasic dopamine release. Capsaicin on the other hand, elicited a 57.0 ± 18.0% increase, which was significantly higher than saline ($W = 52.0$, $p = 0.045$). Saline applied for the first time in the alternate eye caused a 2.1 ± 14.7% increase. Finally, capsaicin resulted in a 35.2 ± 21.8% increase in evoked phasic dopamine release, which did not result in significance when compared to saline in the same cornea.

Figure S7

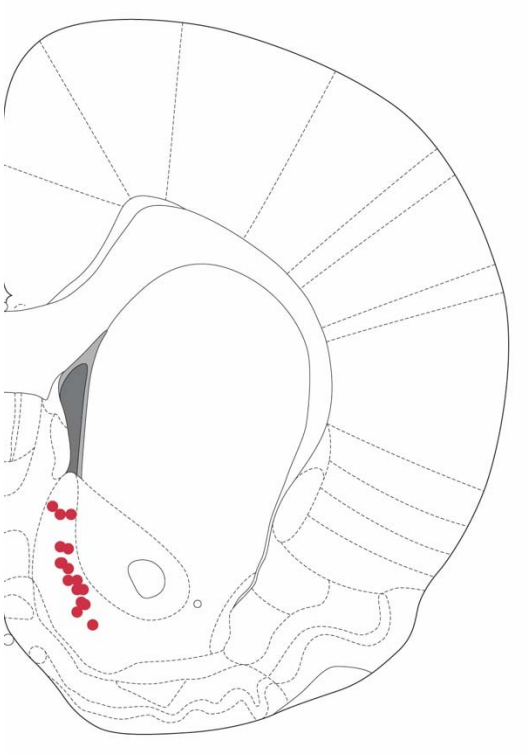

Figure S7. Representative NAc shell recording locations included in tonic and phasic dopamine analysis.

Table S1 . Time to dopaminergic minimum during the first three minutes of each $FSCAV_{tonic}$ recording.

	Mean ± SEM (min.)
Baseline	1.89 ± 0.17
Saline (cornea 1)	1.16 ± 0.24
Cap 1 (cornea 1)	1.36 ± 0.23
Cap 2 (cornea 1)	1.80 ± 0.25
Saline (cornea 1)	1.90 ± 0.29
Cap 1 (cornea 2)	1.16 ± 0.12
Cap 2 (cornea 2)	1.60 ± 0.23

n = 10 rats

References

[1] Molochnikov I, Cohen D. Hemispheric differences in the mesostriatal dopaminergic system. Front Syst Neurosci 2014;8:1–14.

[2] Vreeland RF, Atcherley CW, Russell WS, Xie JY, Lu D, Laude ND, Porreca F, Heien ML. Biocompatible PEDOT:Nafion composite electrode coatings for selective detection of neurotransmitters in vivo. Anal Chem 2015;87:2600–2607.