Supporting Information

On-Column Dimethylation with Capillary Liquid Chromatography-Tandem Mass Spectrometry for Online Determination of Neuropeptides in Rat Brain Microdialysates

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Mass Spectrometry

Table S1. Selected Ions for Fragmentation and Quantitation of Neuropeptides

Peptide	Abbreviation	Precursor m/z[a]	Daughter m/z[b]
Dimethyl leu-enkephalin	Light LE	584.30	425 + 453
$^{2}H_4$ Dimethyl leu-enkephalin	Heavy LE	588.30	429 + 457
Dimethyl yaGfl	Light yaGfl	598.30	439 + 467
$^{2}H_4$ Dimethyl yaGfl	Heavy yaGfl	602.30	443 + 471
Dimethyl met-enkephalin	Light ME	602.30	425 + 453
$^{2}H_4$ Dimethyl met-enkephalin	Heavy ME	606.30	429 + 457

[a]Isolation widths were 3 m/z
[b]Tolerance was ± 0.5 m/z.

Table S2 Selected Ions for Fragmentation and Quantitation in Labeling Tests

Peptide	Center m/z	Isolation[a] Width (m/z)	Daughter m/z
Leu-enkephalin (LE)	556.60	3	397 + 425
yaGfl	570.25	3	411 + 439
Met-enkephalin (ME)	574.20	3	397 + 425
Light LE	588.30	8	425 + 453
Heavy LE	588.30	8	429 + 457
Light yaGfl	602.30	10	439 + 467
Heavy yaGfl	602.30	10	443 + 471
Light ME	602.30	10	425 + 453
Heavy ME	602.30	10	429 + 457

[a]Isolation widths were chosen so that multiple analytes, including potential cross-labeled products, could be detected in a single fragmentation step.

Microdialysis Probe Location

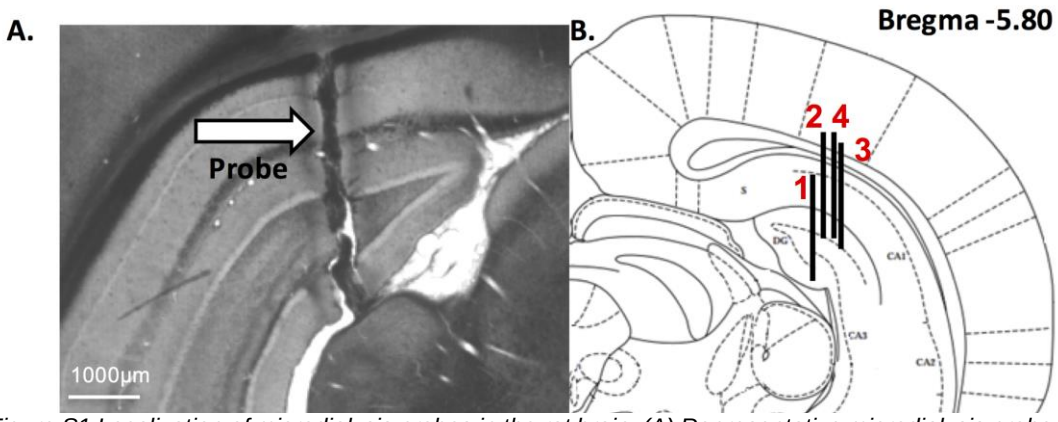

Figure S1 Localization of microdialysis probes in the rat brain. (A) Representative microdialysis probe track. (B) Schematic reconstructions of positions of the four microdialysis probes (adapted from Paxinos and Watson[1]). Coordinates were chosen to sample from the CA1 region. Note that probe 1 is in the dentate gyrus. Numbers in red correspond to the rat numbers in Table 3 of the text.

Labeling Tests

To confirm the absence of unlabeled or cross-labeled peptides, we performed a series of tests in which labeling was conducted as described in the text but: 1) The Ringer's standard was injected prior to treatment with labeling reagents and no aqueous standard was injected and 2) The aqueous standard was injected after quenching of the light label but before treatment with the heavy label. In this test, no Ringer's standard was injected. For each test the Ringer's standard consisted of 50 pM (Fig. S2), 100 pM (Fig. S3), or 200 pM (Fig. S4) LE, yaGfl, and ME in Ringer's and the aqueous standard consisted of matching concentrations (either 50, 100, or 200 pM) of LE, yaGfl, and ME in water. As expected, for the first test we observed only light product in the mass spectra and for the second test we saw only heavy product, indicating complete labeling.

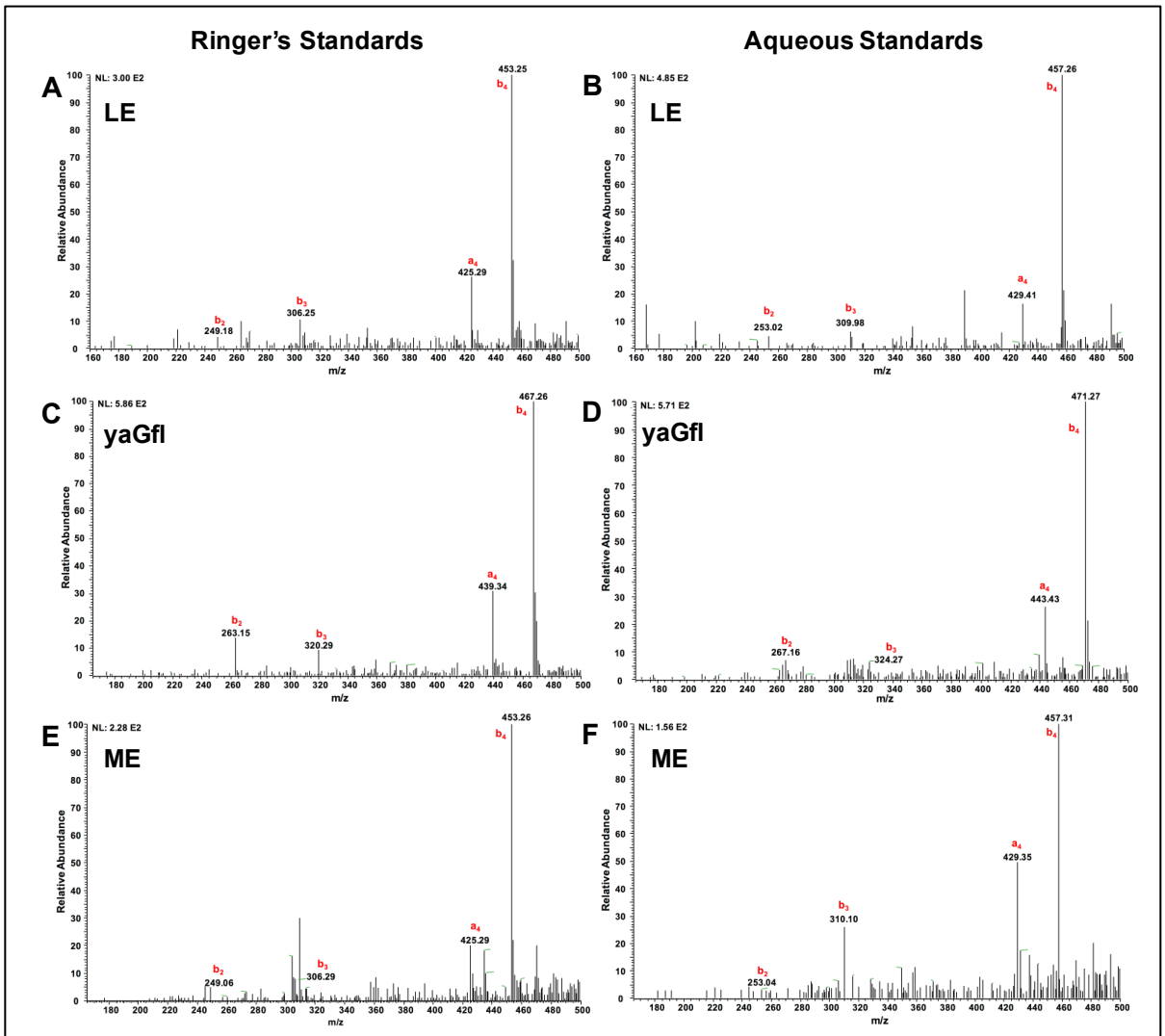

Figure S2 Labeling test results for 50 pM Ringer's standards (left column) and 50 pM aqueous standards (right column) for A-B) LE, C-D) yaGfl, and E-F) ME. Peak identifications were made using prospector.ucsf.edu/prospector/cgi-bin/mssearch.cgi.

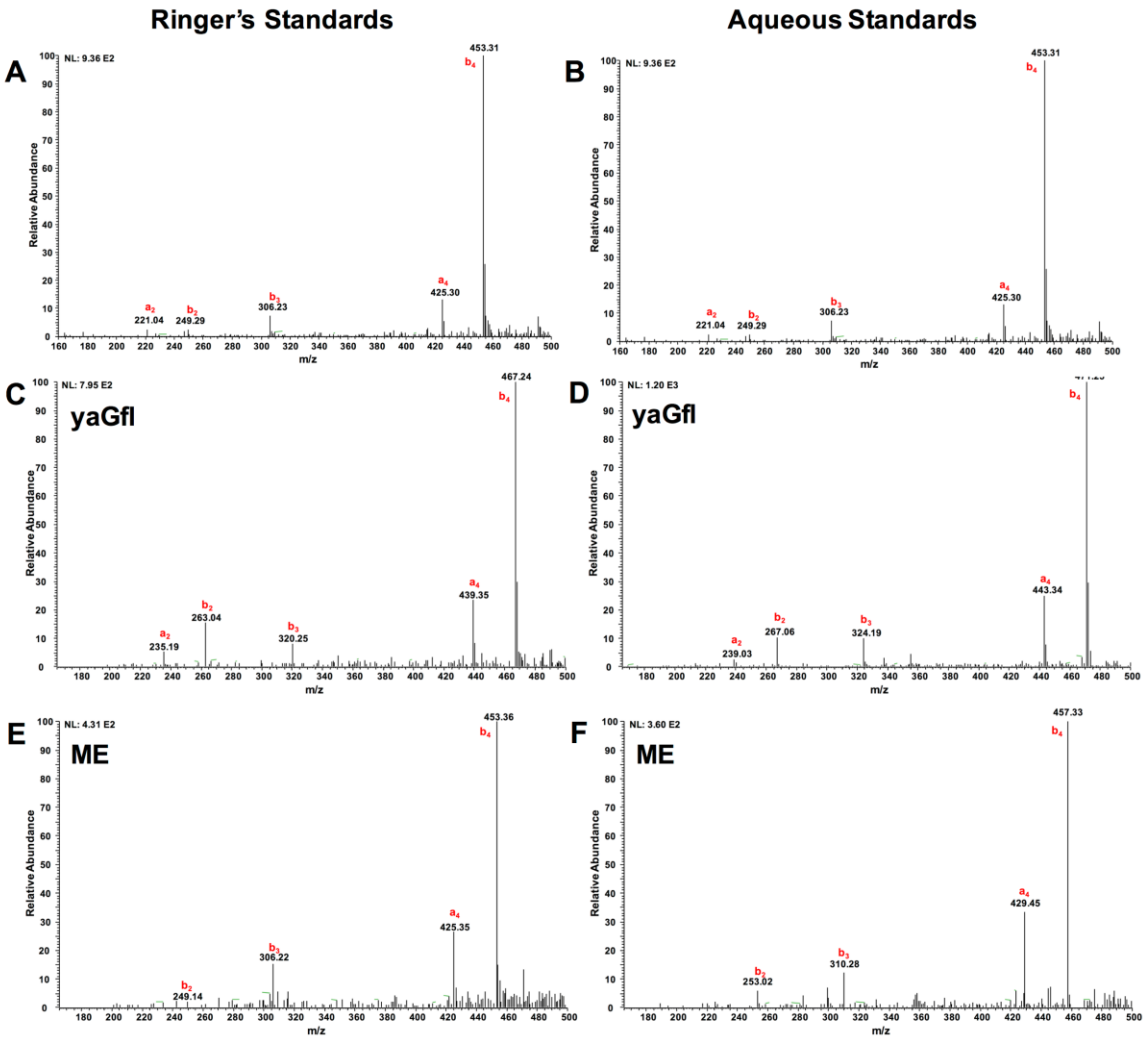

Figure S3 Labeling test results for 100 pM Ringer's standards (left column) and 100 pM aqueous standards (right column) for A-B) LE, C-D) yaGfl, and E-F) ME.

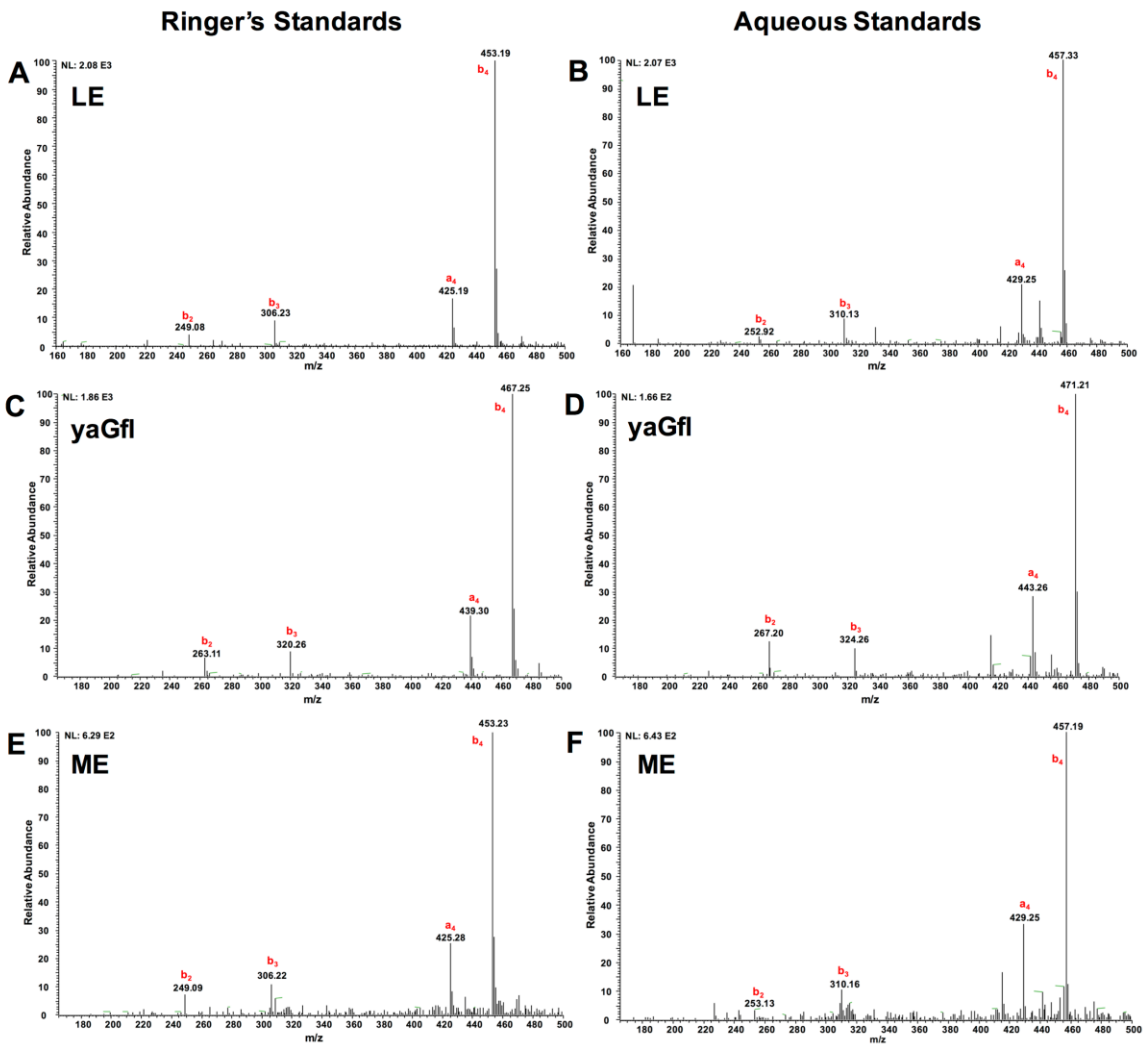

Figure S4 Labeling test results for 200 pM Ringer's standards (left column) and 200 pM aqueous standards (right column) for A-B) LE, C-D) yaGfl, and E-F) ME.

In Vivo Microdialysis Data

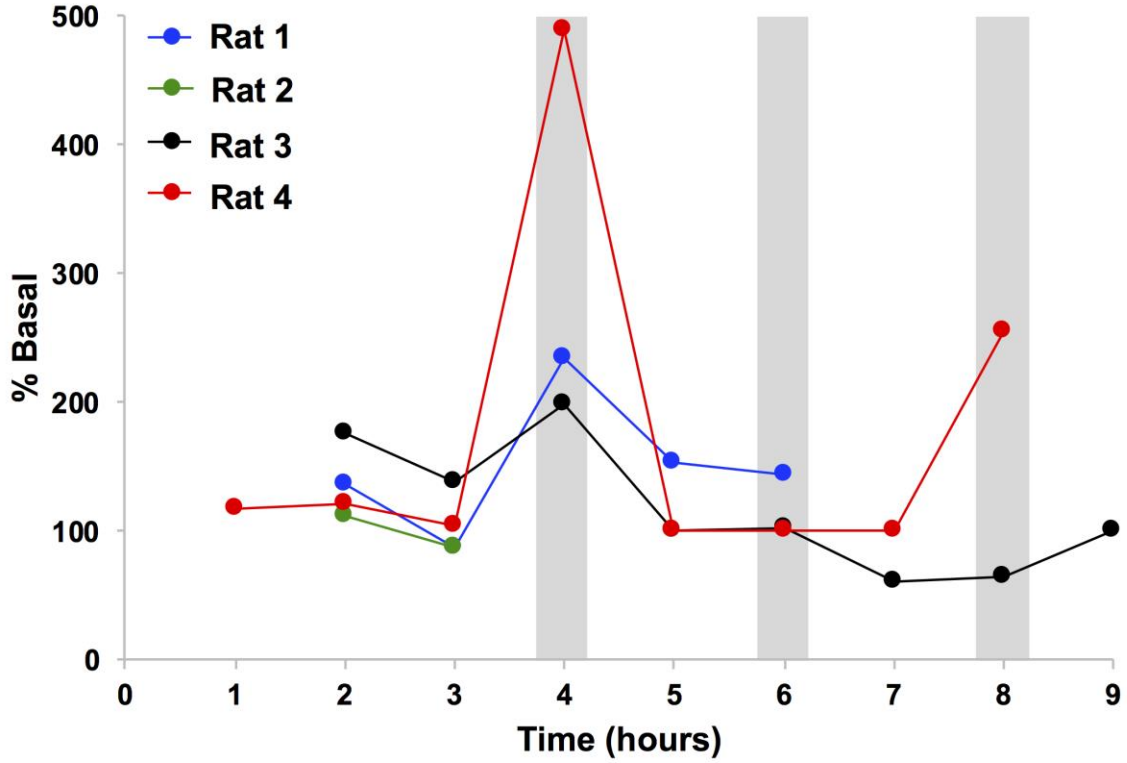

Figure S5 Measured LE Levels for Each Rat. To determine the percent basal, all detectable basal light LE peak areas were divided by the corresponding heavy peak area to obtain the relative area. The basal relative areas for each individual rat were averaged. Each time point was divided by the mean basal relative area and expressed as a percentage. Gray shaded areas indicate points that were collected during potassium stimulation. The time axis of each rat has been adjusted so that the potassium stimulated points are aligned.

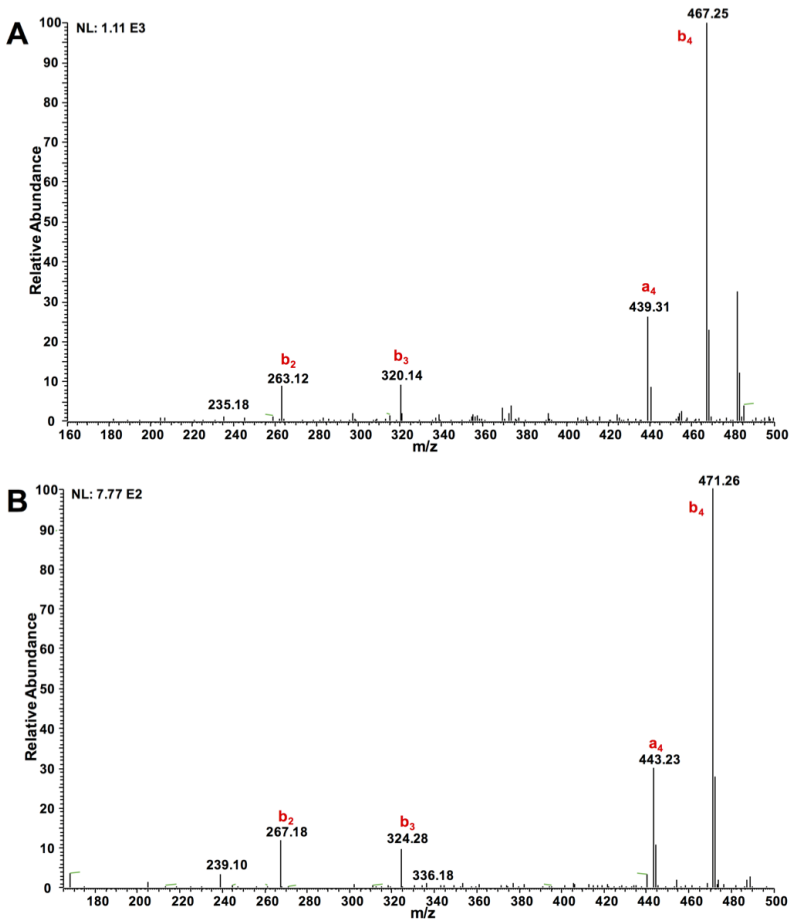

Figure S6 Mass spectra at the point of elution for A) light yaGfl and B) heavy yaGfl during 10-minute stimulation with 100 mM KCl Ringer's. Corresponding chromatograms can be found in Figure 5.

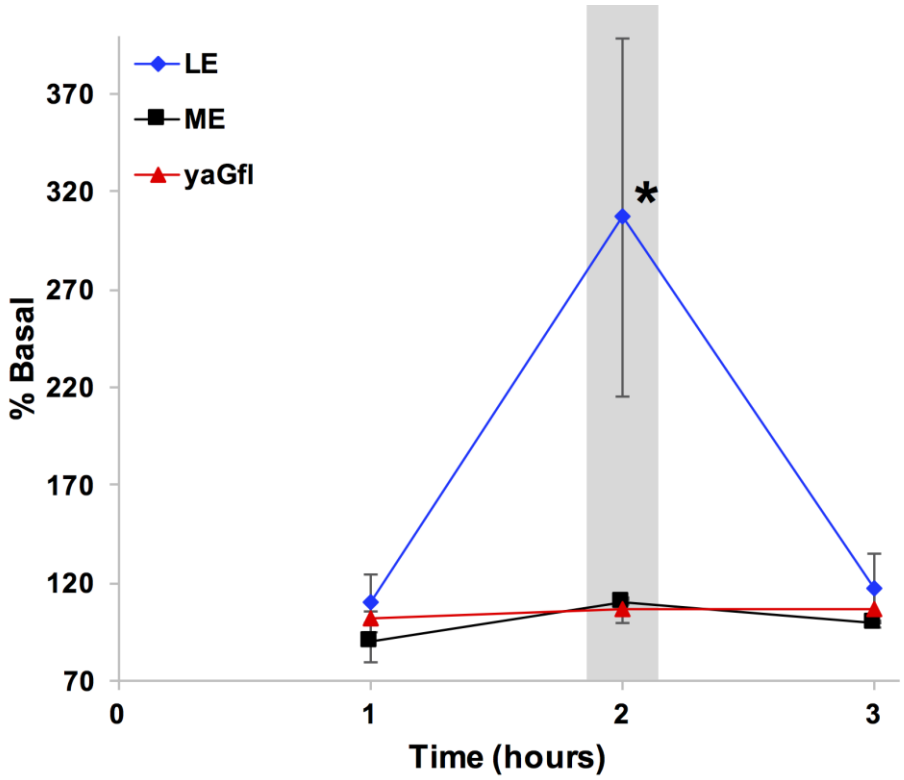

Figure S7 Comparison of LE, ME, and yaGfl Potassium Stimulated Levels to Basal Levels. Percent basal levels were determined by dividing the relative area (light peak area/ heavy peak area) at each time point by the mean basal relative area for each rat, which was then expressed as a percentage. The shaded gray area indicates points that were collected during potassium stimulation. The time axes have been adjusted so that the potassium stimulated points are aligned. The asterisk indicates a statistically significant (p=0.03) increase in LE levels during potassium stimulation as determined using a one-tailed t-test (with 95% confidence). The error bars represent the SEM of n = 3 rats.

Table S3 Comparison of Basal[a] Levels of ME with Potassium Stimulated[b] Levels

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	P-value
Between Groups	0.015	1	0.015	0.20	0.67
Within Groups	0.71	9	0.079		
Total	0.72	10			

[a]The mean of pooled basal levels (± SEM) was 0.260 ± 0.062 for n = 6 measurements.
[b]The mean of pooled potassium stimulated levels (± SEM) was 0.33 ± 0.10 for n = 5 measurements.
[c]Comparisons were made using a one-tailed t-test with 95% confidence.

Citations

(1) Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*; Academic Press, 1998.