Electronic Supplementary Material (ESI) for ChemComm

Gauging circadian variation in ketamine metabolism by real-time breath analysis

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Experimental section

Secondary Electrospray Ionization-Mass Spectrometry (SESI-MS)

The front-end of a quadrupole time-of-flight mass spectrometer (QqTOF-MS; Sciex Tripletof 5600+) was modified to feature a lab-built secondary electrospray ionization (SESI; Ref. 7 in the main text) device. The SESI chamber consisted of a gas tight cylinder welded to the curtain plate. The cylinder had two ports: one for the entrance of sample gas and the second one for the exhaust. In this way, the gas sample crossed an electrospray plume generated co-axially to the sampling orifice of the MS. The electrospray was formed by infusing H_2O 0.1% formic acid through an uncoated silica capillary (PicoTip emitter - O.D. 360 µm – I.D. 20 µm). The flow was established at a constant flow rate of ~200 nL/min by pressurizing the water reservoir. The liquid was electrified by using the high voltage power supply of the mass spectrometer. The measured electrospray current was in the range of 100 nA. The water electrospray was operated in the cone-jet mode as described by elsewhere[1]. Formation of a stable cone-jet was verified by visual inspection with a magnifier. Hydronium ions produced in the electrospray plume underwent a proton transfer reaction with neutral vapor analytes[2] stemming from the mouse.

Animals and experimental methods

Group-housed adult male adult mice were used in this study. Mice were held in standard laboratory conditions with food and water available *ad libitum* and exposed to 12 hours of light per day (LD 12:12). For at least 3 weeks before experiments, mice were either kept in "normal" LD with lights on from 07:00 to 19:00 or "inverted" 12-h shifted LD cycle (i.e., lights on from 19:00 to 07:00). All measurements were recorded between 17:00 and 21:00 for approximately 1 hour/mouse, i.e., morning for "normal" mice or evening for "inverted" mice.

All experimental animals originated from B6.129S4(Cg)-*Arntl*^{tm1Weit}/J (*Bmal1*$^{fl/fl}$) and B6.Cg-Tg(*Alb-cre*)^{21Mgn}/J (*Alb-Cre*$^{+/-}$)mice and were bred in our colony at the University of Zurich as offspring to *Bmal1*$^{fl/fl}$ x *Bmal1*$^{fl/fl}$;*Alb-Cre*$^{+/-}$ breeders. Mice were genotyped for *Bmal1* and *Alb-Cre* allels as described previously (Ref. 4 in the main text). For simplicity, *Bmal1*$^{fl/fl}$ mice with two intact copies of the *Bmal1* gene and no further transgene (i.e., lacking an allele of Alb-Cre) are referred to as wild-type (WT) mice, and *Bmal1*$^{fl/fl}$ mice also having an allele of Alb-Cre are described as knock-out (KO) mice because they lack BMAL protein in the liver and therefore have a non-functional liver clock. All animal experiments were in accordance with the Helsinki Declaration and approved by the veterinary office of the Canton of Zurich.

At the indicated times during the light/dark cycle, mice were intraperitoneally (i.p.) injected with 30 mg/kg (in 100 µl phosphate buffered saline) ketamine (Ketalar, Pfizer) and immediately placed inside a clear plexiglass chamber upstream the SESI source (Fig. S1). The mouse chamber was constantly flushed with 2 L/min of compressed "medical air" delivered via a teflon tube. The

vapors emitted by the mouse were monitored in real-time. The mass spectra were acquired in positive ion mode in full MS mode covering a mass range of 100-400 m/z. Accumulation time was set to 5 s.

Data analysis

Raw *.wiff mass spectra (Sciexs proprietary format) was converted into *.mzXML format using Proteowizard[3]. The *.mzXML were then imported in MATLAB (version R2016a). The mass spectra were read and linearly interpolated ($6x10^5$ points in the mass range m/z 100-400). The mass spectra were centroided (threshold intensity = 100 a.u.). Time profiles for each m/z values in the resulting peak list were obtained by summing the intensity of each peak +/- 0.002 Da window. The raw time profiles were smoothed using a robust version of local regression using weighted linear least squares and a first degree polynomial model. Some selected time traces displaying a distinct different trend between different circadian phases were subjected to hierarchical cluster analysis (Ward linkage method; cosine distance metric; Fig. 3).

Fig. S1. Experimental set-up. The vapors emitted by a mouse after being injected with ketamine were constantly sampled using 2 L/min of medicinal air through the mouse chamber. Gas-phase analytes (i.e., ketamine and its metabolites) entered the SESI source attached to the curtain plate of the mass spectrometer. Full mass spectra were recorded in the range m/z 100-400, allowing to capture pharmacokinetic profiles in real-time with a time resolution of 5 s.

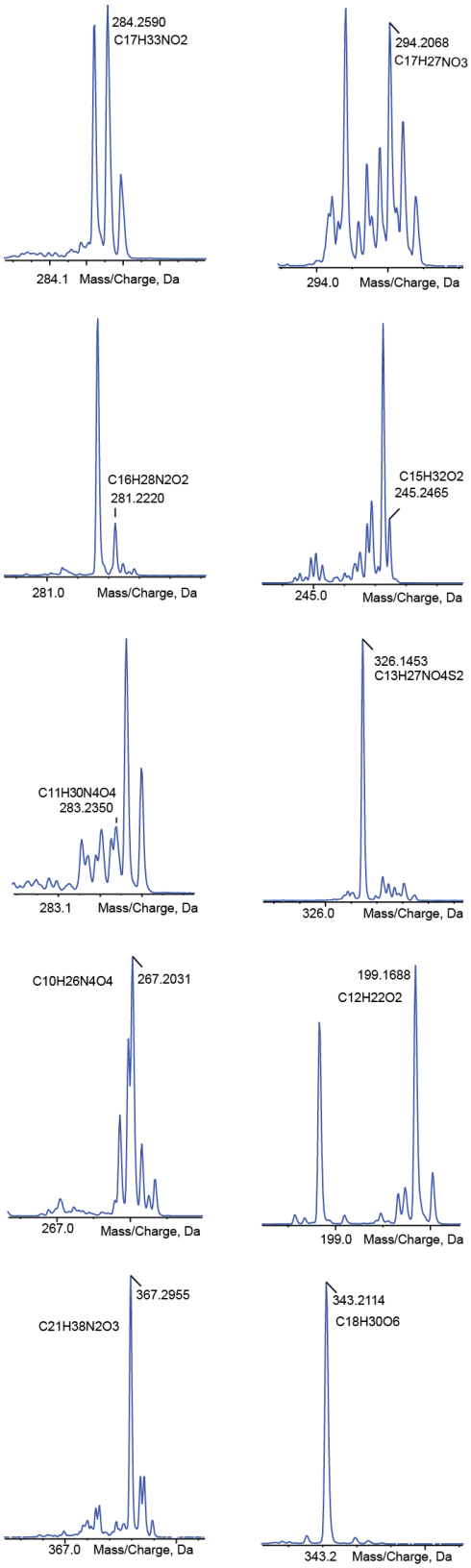

Fig. S2. Representative mass spectra of the features reported in Fig. 3 in the main text.

Cluster	m/z	Molecular formula	RDBE	error (ppm)
1	284.2590	$C_{17}H_{33}NO_2$	2	2.2
1	285.2113	$C_{10}H_{28}N_4O_5$	-1	-6.8
1	283.2350	$C_{11}H_{30}N_4O_4$	-1	3.4
1	281.2220	$C_{16}H_{28}N_2O_2$	4	-1.3
1	279.2034	$C_{11}H_{26}N_4O_4$	1	2.5
1	267.2031	$C_{10}H_{26}N_4O_4$	0	1.5
1	351.3005	$C_{21}H_{38}N_2O_2$	4	-0.4
1	367.2955	$C_{21}H_{38}N_2O_3$	4	0.0
1	311.2329	$C_{17}H_{30}N_2O_3$	4	-0.1
1	125.0255			
1	123.0542	$C_6H_6N_2O$	5	-9.0
2	294.2068	$C_{17}H_{27}NO_3$	5	1.6
2	261.2462			
2	248.2580	$C_{14}H_{33}NO_2$	-1	-1.7
2	245.2465	$C_{15}H_{32}O_2$	0	-4.2
2	326.1453	$C_{13}H_{27}NO_4S_2$	1	-0.4
2	199.1688	$C_{12}H_{22}O_2$	2	-2.2
2	378.2487	$C_{18}H_{35}NO_7$	2	0.1
2	198.1263	$C_{14}H_{15}N$	8	-7.1
2	284.2961	$C_{18}H_{37}NO$	1	4.4
2	343.2114	$C_{18}H_{30}O_6$	4	-0.3
2	329.1929	$C_{13}H_{24}N_6O_4$	5	-0.9
2	361.2219	$C_{18}H_{32}O_7$	3	-0.5
2	327.1798	$C_{17}H_{26}O_6$	5	-1.3

Table S1. Accurate masses, proposed molecular formulae (assuming protonated species), corresponding Ring Double Bond Equivalents (RDBE) and mass error (ppm) for the 24 features reported in Fig 3 in the main text.

References

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3 D. Kessner, M. Chambers, R. Burke, D. Agus and P. Mallick, *Bioinformatics*, 2008, **24**, 2534.