SI Results

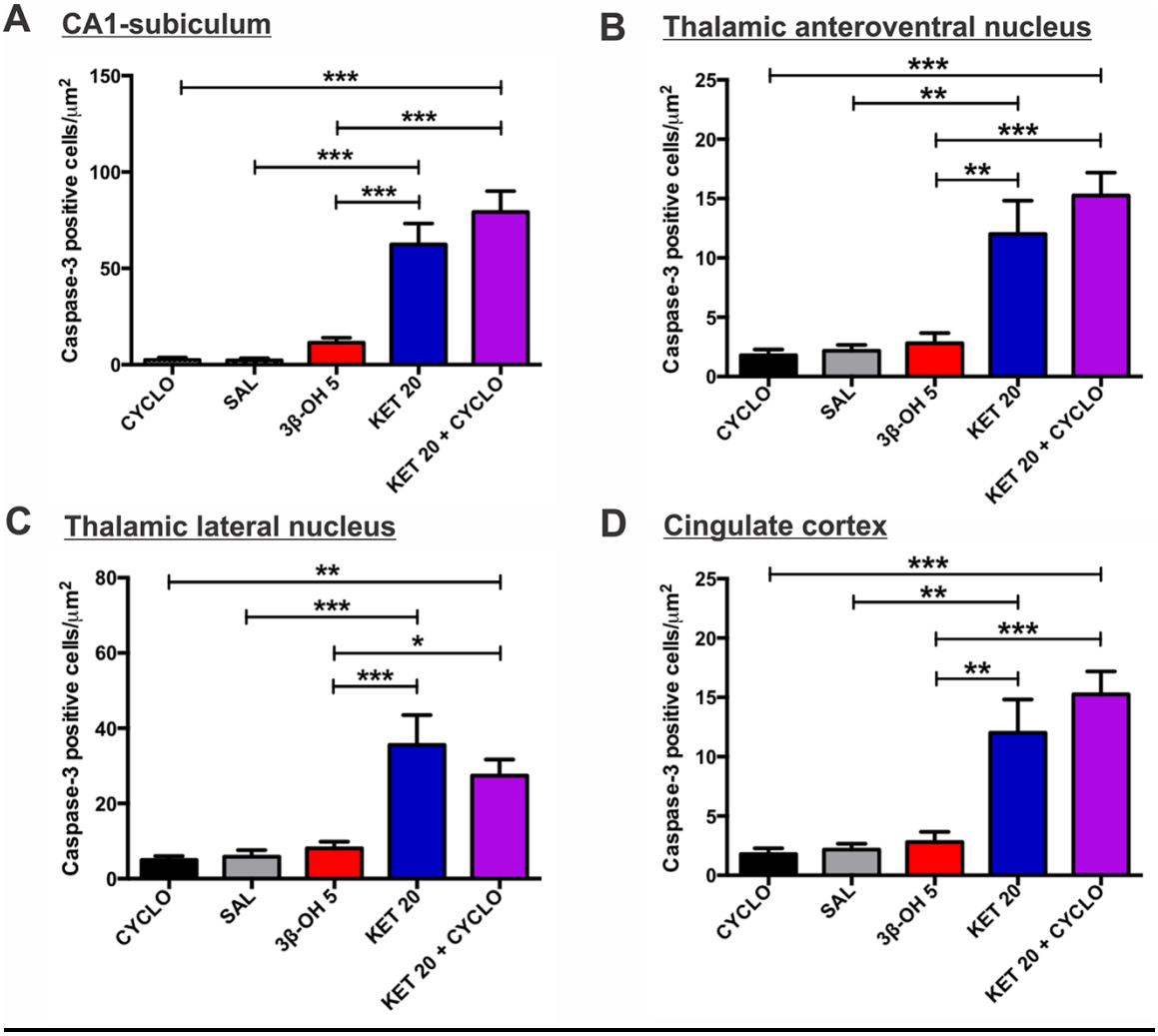

Figure S1: 3β-OH, unlike ketamine, does not cause developmental neuroapoptosis at lower doses. 3β-OH (at 5 mg kg^{-1}, i.p., 3β-OH 5), ketamine (at 20 mg kg^{-1}, i.p., KET 20) and ketamine (at 20 mg kg^{-1}) + β-cyclodextrin (KET 20 + CYCLO) were injected at P7 every 2 hrs for total of 6 doses. **A.** In CA1-subiculum region, there was an approximate 29- and 32-fold higher density of caspase-3 stained neurons compared to vehicle controls (***, $p < 0.001$ vs. SAL and $p < 0.001$ vs. CYCLO). Compared to ketamine alone, the

ketamine+β-cyclodextrin group showed a non-signficant increase in caspase-3 activation ($p = 0.449$). When either ketamine alone or ketamine+β-cyclodextrin groups were compared to 3β-OH, there was a significant increase in caspase-3 activation (***, $p < 0.001$ vs. KET 20 and $p < 0.001$ vs. KET 20+CYCLO) suggesting that 3β-OH, unlike ketamine, does not cause significant developmental neuroapoptosis in P7 rat pups. Activated caspase-3 staining in 3β-OH-treated animals compared to saline or β-cyclodextrin controls was not significantly increased ($p = 0.876$ vs. SAL and $p = 0.889$ vs. CYCLO) (n = 6 pups per data point). **B.** In thalamic anteroventral nucleus (TAV) there was minimal caspase-3 activation in vehicle groups, while there was a significant increase in caspase-3 activation in the ketamine (KET 20, **, $p = 0.002$) or ketamine+β-cyclodextrin (KET 20 + CYCLO) groups (***, $p < 0.001$). The level of caspase-3 activation in 3β-OH animals was comparable to vehicle controls (CYCLO, SAL) and significantly lower than either in the ketamine (**, $p = 0.003$) or ketamine+β-cyclodextrin (***, $p < 0.001$) groups (n = 6 pups per data point). **C.** In thalamic lateral nucleus (TL) there was minimal caspase-3 activation in the vehicle groups (CYCLO, SAL), while there was significant caspase-3 activation in the ketamine (KET 20) or ketamine+β-cyclodextrin (KET 20 + CYCLO) groups (***, $p < 0.001$ and **, $p = 0.008$). The level of caspase-3 activation in 3β-OH animals was comparable to vehicle controls and significantly lower than in the ketamine (***, $p < 0.001$) or ketamine+β-cyclodextrin (*, $p = 0.025$) groups (n = 6 pups per data point). **D.** In the cingulate cortex, the ketamine+β-cyclodextrin group (KET 20 + CYCLO) exhibited a significant increase in caspase-3 activation compared to vehicle control (CYCLO) (***, $p < 0.001$). The level of caspase-3 activation in 3β-OH treated animals was comparable to vehicle controls ($p = 0.999$ vs. SAL; $p = 0.995$ vs. CYCLO) and significantly

lower than the ketamine+β-cyclodextrin group (***, $p < 0.001$) (n=6 pups per data point). All statistical analyses were done using one-way ANOVA with Tukey's post-hoc test.

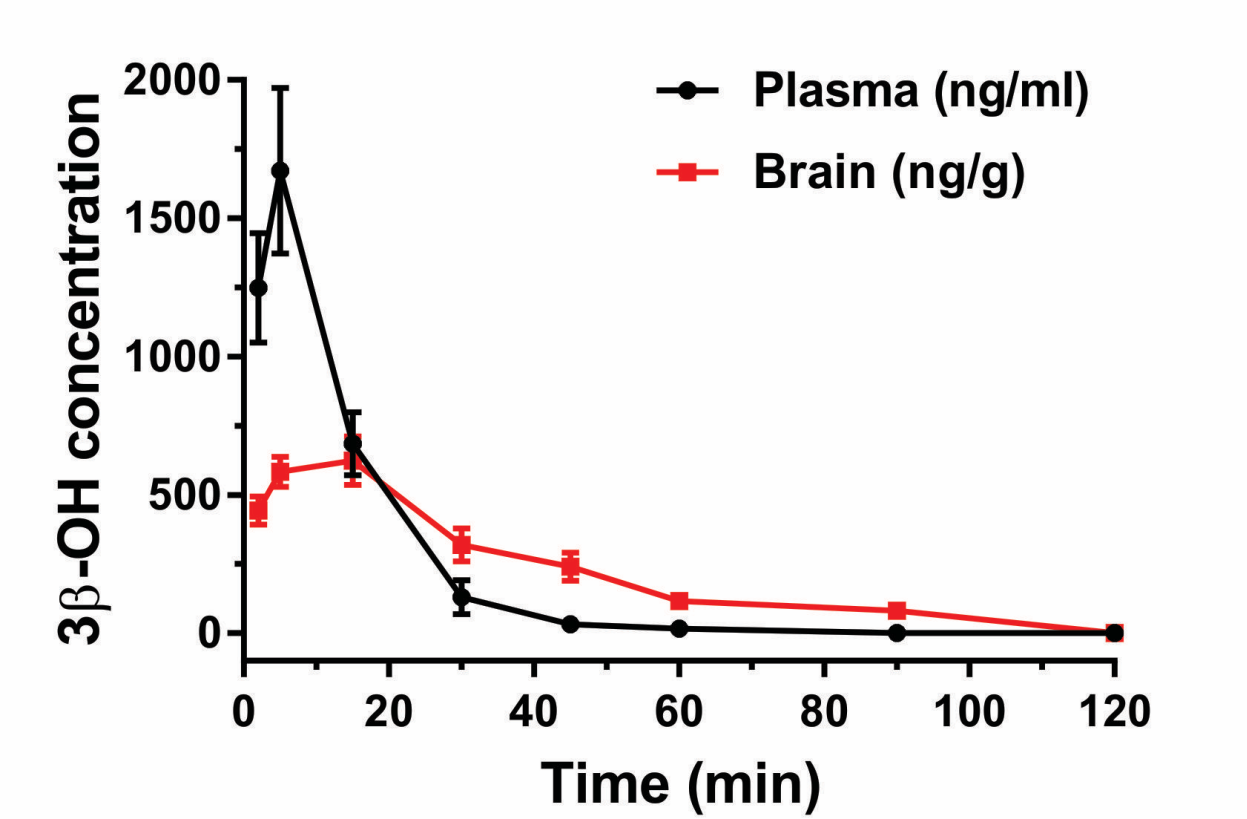

Figure S2: Plasma and brain homogenate concentrations of 3β-OH. The *in vitro* plasma and brain concentrations in P7 rat pups treated with 3β-OH at 10 mg kg^{-1}, i.p. were determined over the course of 120-min after a single 3β-OH injection. The peak in plasma and brain homogenate occurred at 5 min and 15 min, respectively, followed by a rapid decrease in plasma levels. 3β-OH was not detectable in plasma after 60 min and in brain tissue after 90 min. The peak plasma concentration at 5 min was 1670 ng ml^{-1} whereas the peak brain concentration at 15 min was 620 ng g^{-1} (n = 6-17 pups per data point).

SI Materials and Methods

Drugs and chemicals. The procedure for step-by-step synthesis of 3β-OH is provided in Supplementary Material **(Fig. S3)** and is an improvement on methods described previously[1]. For *in vivo* experiments 3β-OH was freshly dissolved in 15% (2-hydroxypropyl)-β-cyclodextrin (Santa Cruz Biotechlogy Inc, USA) solution (vehicle) and injected i.p. at doses from 1 to 100 mg kg^{-1}; ketamine (Sigma-Aldrich Chemicals, St. Louis, MO, USA) was dissolved in saline and injected i.p. at doses from 1 to 140 mg kg^{-1}. All steroids for electrophysiology experiments were prepared as 3 mM stock solutions in dimethylsulfoxide (DMSO) and freshly diluted to the final concentrations in the external solution at the time of experiments.

Assessment of the hypnotic effects of ketamine and 3β-OH - loss of righting reflex (LORR). To assess LORR, rat pups were turned onto their backs upon injection of the desired drug and anaesthesia/'unresponsiveness' was defined as the inability to turn back over onto all 4 legs ("right themselves") within 5-10 sec. The return of righting reflex was recorded when the animals were able to right themselves up onto all four paws. Immediately after administration of anaesthesia, pups were reunited with their mothers and allowed to nurse. The ED_{50} was calculated using the Hill-Langumir equation as follows: $PE([3\beta\text{-OH}]) = PE_{max} / (1+(ED_{50} / [3\beta\text{-OH}])^{h})$ where PE_{max} is the maximal percent of animals with LORR, ED_{50} is the dose that produces 50% effect, and *h* is the apparent Hill-Langmuir coefficient that defines the slope of the curve. The fitted values are reported with > 95% linear confidence limits. The LD_{50} was calculated using the Hill-

Langumir equation as follows: PD ([3β-OH]) = PD_{max} / (1+(LD_{50} / [3β-OH])^{h}) where PD_{max} is the maximal percent of dead animals, LD_{50} is the dose that produces 50% effect, and *h* is the apparent Hill-Langmuir coefficient that defines the slope of the curve. The fitted values are reported with > 95% linear confidence limits.

Morphological assessment of developmental neuroapoptosis. All rat pups were deeply anaesthetized using isoflurane, and then perfused with 4% paraformaldehyde in phosphate buffer (0.1M), pH 7.4 for immunohistochemistry studies at 2 hr after anaesthesia. For activated caspase-3 staining, 50-μm-thick vibratome sections were washed in 0.01 M phosphate-buffered saline (PBS), quenched for 10 min in a solution of methanol containing 3% hydrogen peroxide, then incubated for 1 hr in blocking solution (5% goat serum/0.1% Tween-20 in PBS). This was followed by incubation overnight with a primary anti-active caspase-3 antibody (Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb (Biotinylated), Cell Signaling Technology) diluted 1:100 in blocking solution (1% goat serum/0.1% tween-20 in PBS) at 4˚C. After incubation with biotinylated antibody, sections were reacted in the dark with a Vectastain ABC reagent (Vectastain ABC HRP Elite Kit (Peroxidase, Standard), Vector Labs). The reaction complexes were developed by incubating in DAB (3,3-diaminobenzidine) substrate (ImmPACT DAB Peroxidase (HRP) Substrate, Vector Labs) following the manufacturer's instructions.

Quantitative immunohistochemistry. To determine the density of activated caspase-3 stained neurones in a given brain region, the sections were first scanned at 20x magnification using an Aperio Scanscope XT digital slide scanner (Leica Biosystems). We examined the brain regions most vulnerable to apoptosis; regions were based on the postnatal rat pup brain atlas[2]: hippocampal CA1-Subiculum junction (-3.80 mm from

bregma); thalamic nuclei: thalamic anterior ventralis (TAV) (-1.60 mm from bregma) and thalamic lateralis (TL) (-1.60mm from bregma); and cingulate cortex (0.20 mm from bregma). The regions were extracted at 400-800 μm scale from scanned sections (.svs file), converted to .tiff files, and spatially calibrated using a 1000 μm^2 grid prior to quantifying using Image-Pro Plus 7.0 software (Media Cybernetics). The number of caspase-3 positive cells were quantified from a single section on both sides, averaged to provide a single data point, divided by the area measured, and multiplied by 1000 to express the densities of caspase-3 neurons per μm^2. The counting was done by an investigator blinded to the experimental conditions. The results from different groups were statistically analyzed by one-way ANOVA using Graph Pad Prism 5.01 software (Graph Pad, San Diego, CA, USA). Data are presented as mean (SEM).

Assessment of cognitive function with Radial Arm Maze (RAM). RAM evaluates spatial learning and reference memory in rodents and can detect subtle changes in learning and memory caused by anesthetics and sedatives. The apparatus consists of eight equally spaced arms radiating from a central platform; a food reward is placed at the end of each arm. For our study the arms were baited with chocolate cereal. Rats exhibit reference memory when they visit an arm containing a food reward. The rats were food-restricted (with free access to water) from P45 to P70 (when RAM testing was completed), to achieve 85%–90% of their *ad libitum* weight. Visual cues were provided to assist spatial navigation. Rats were tested until they reached criterion; that is, to give 8 correct responses (retrieve a food reward placed in each arm) out of the first 9 responses for 4 consecutive days of testing. Statistical analysis was performed using t-tests for predetermined pairwise comparisons (Fig. 3B and C). Fisher's exact test was

used to compare acquisition rates of different treatment groups (Fig. 3D). Using the standard version of GraphPad 5.01 software we considered $p<0.05$ to be statistically significant. The experimenters were blinded to the experimental condition.

High-performance liquid chromatography (HPLC)-tandem mass spectrometry (LC-MS/MS) for the quantification of 3β-OH [(3β,5β,17β)-3-hydroxyandrostane-17-carbonitrile] in P7 rat EDTA plasma and brain tissue. We measured 3β-OH in plasma, and brain tissue of P7 rat pups. For plasma, we added 400 µL methanol/ 0.2 mM $ZnSO_4$ (70/30, v/v; protein precipitation solution) to 100 µL plasma samples. Epipregnanolone (1 µg mL^{-1} final concentration) was added as an internal standard to the protein precipitation solution. For brain samples, tissue was homogenized in 1 mL acetonitrile/PBS (1:1, v v^{-1}) using a Bullet Blender tissue homogenizer (Next Advance) followed by addition of epipregnanolone (at 1 µg mL^{-1} final concentration) as an internal standard. Plasma and brain samples were then vortexed and centrifuged (16,000 *g*, 15 min, 4˚C) and transferred into 1.5 mL glass HPLC vials (Phenomenex) and 10 µL of the supernatant was injected onto the analytical HPLC column (Eclipse XDB-C8, 4.6 x 150 mm, 3.5 µm, Agilent Technologies, Palo Alto, CA, USA). The mobile phases consisted of A: 0.1% formic acid and B: methanol. The following gradient was run: 0 to 1 min with 60% B to 99% B, from 1 to 9 min % B was held 99%, after which the column was re-equilibrated to the starting conditions for 1 min. The flow rate was 1 mL min^{-1} and the column was kept at 30ºC.

The HPLC system (Agilent 1200 components, Agilent Technologies) was interfaced with an API5500 QTRAP mass spectrometer (Sciex, Concord, ON, Canada) using an atmospheric pressure chemical ionization (APCI) source. The mass spectrometer was run in positive multiple reaction monitoring (MRM) mode. Peak area

ratios obtained from MRM mode of the mass transition for 3β-OH (m/z= 284.5→130.2 (quantifier transition; declustering potential (DP): 140V, entrance potential (EP): 8V, collision energy (CE): 55V, collision cell exit potential (CXP): 12V) and m/z= 284.5→72 (qualifier transition)) and the internal standard epipregnanolone (m/z= 301.5→269.2) were used for quantification.

The following mass spectrometer parameters were used: collision gas: medium, curtain gas: 20 L min^{-1}, ion source gases 1 and 2: 25 L min^{-1}, ion spray voltage: 5500V, temperature: 500˚C. The method was linear from 75 ng mL^{-1} to 10 µg mL^{-1} in plasma and brain, respectively. Quality control of the assay consisted of randomly distributed quality control samples with the concentrations of 0.15, 0.75 and 7.5 ng mL^{-1} 3β-OH.

Pharmacokinetic (PK) analysis. PK parameters were computed from drug concentration-time data using a non-compartmental model as implemented in the WinNonlin Phoenix software (version 7.0, Certara, Princeton, NJ, USA).

Brain slice preparation for patch-clamp electrophysiology experiments. Animals were anaesthetized briefly with isoflurane, decapitated, and their brains rapidly removed. Fresh horizontal brain slices, 250-300 µm thick, were sectioned at 4°C in pre-chilled solution containing (in mM): sucrose 260, D-glucose 10, $NaHCO_3$ 26, NaH_2PO_4 1.25, KCl 3, $CaCl_2$ 2, $MgCl_2$ 2, using a vibrating micro slicer (Leica VT 1200S). Brain slices were immediately incubated for 45 min in a solution containing (in mM): NaCl 124, D-glucose 10, $NaHCO_3$ 26, NaH_2PO_4 1.25, KCl 4, $CaCl_2$ 2, $MgCl_2$ 2 at 37°C prior to use in electrophysiology experiments, which were conducted at room temperature. During

incubation, slices were constantly perfused with a gas mixture of 95 % O_2 and 5 % CO_2 (v/v).

Electrophysiology Recordings. The external solution for whole-cell recordings consisted of (in mM): NaCl 125, D-glucose 25, $NaHCO_3$ 25, NaH_2PO_4 1.25, KCl 2.5, $MgCl_2$ 1, $CaCl_2$ 2. This solution was equilibrated with a mixture of 95 % O_2 and 5 % CO_2 (v/v) for at least 30 min with a resulting pH of approximately 7.4. For current-clamp experiments, the internal solution consisted of (in mM): potassium-D-gluconate 130, EGTA 5, NaCl 4, $CaCl_2$ 0.5, HEPES 10, Mg ATP 2, Tris GTP 0.5, pH 7.2. For recording of IPSCs, we used an internal solution containing the following (in mM): KCl 130, NaCl 4, $CaCl_2$ 0.5, EGTA 5, HEPES 10, $MgATP_2$ 2, Tris-GTP 0.5, and lidocaine *N*-ethyl bromide (QX-314) 5. pH was adjusted with KOH to 7.25. For recordings of EPSCs, this internal solution was modified by replacing KCl with equimolar K-gluconate and external solution contained 0-0.5 mM $MgCl_2$. Glass micropipettes (Sutter Instruments O.D. 1.5 mm) were pulled using a Sutter Instruments Model P-1000 and fabricated to maintain an initial resistance of 3-5 MΩ. Neuronal membrane responses were recorded using a Multiclamp 700B amplifier (Molecular Devices, Foster City, CA). Voltage current commands and digitization of the resulting voltages and currents were performed with Clampex 8.3 software (Molecular Devices) running on an IBM-compatible computer. Resulting current and voltage traces were analyzed using Clampfit 10.5 (Molecular Devices, Foster City, CA, USA). Statistical analysis was performed using two-tailed paired t-test where appropriate, as well as one-way ANOVA; significance was accepted as $p<0.05$. Where applicable, Tukey's test for post hoc comparison was also used. Statistical and graphical

analyses were performed using GraphPad Prism 5.01 software and Origin 7.0 (OriginLab, Northhampton, MA, USA).

Current-clamp experiments. To assess hyperpolarization-induced (rebound) firing of thalamic and subicular neurones, a multi-step protocol was used. We injected a depolarizing current of 200 pA, followed by a series of hyperpolarizing currents in 50 pA increments stepping from -200 to -400 pA in the presence of synaptic blockers [(20 μM picrotoxin, 50 μM D-2-amino-5-phosphonovalerate (d-APV), and 10 μM 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX)] in the external solution. Subsequent resting membrane potentials, rebound action potentials and input resistances were determined. Resting membrane potential was measured at the beginning of each recording and was not corrected for the liquid junction potential, which was between 5 and 10 mV in our experiments. The membrane input resistance was calculated by dividing the steady-state hyperpolarizing voltage deflection by the injected current.

Spontaneous IPSCs. In the patch-clamp configuration at a holding potential of −70 mV, action potential-dependent (spontaneous) $GABA_A$-mediated currents (sIPSCs) were isolated in the presence of the NMDA channel blocker d-APV (50 μM) and AMPA channel blocker NBQX (10 μM). To confirm we indeed recorded sIPSCs, 20 μM picrotoxin was added at the end of several recordings. All data were analyzed using MiniAnalysis software (Synaptasoft). The limits for sIPSCs were set in most of recordings at 3× the root mean square of baseline noise. In our analysis of kinetics of spontaneous synaptic currents, we included only isolated (i.e., nonoverlapping) events. All sIPSCs were

analyzed with respect to peak amplitude and decay time course, which was analyzed only in case of near perfect fit, using a single exponential function. Plots describing the cumulative distribution of inter-event interval function of sIPSC properties were derived empirically using GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA). In brief, these plots were derived from all recorded neurons, and they represent a portion of events whose apparent inter-event intervals were less than a certain value.

Evoked synaptic currents. Evoked synaptic transmission was evaluated by stimulation within the CA1 alveus/stratum oriens region, and the responses were recorded within patch-clamped subicular neurones. Synaptic stimulation was achieved using a constant current isolated stimulator DS3 (Digitimeter). Properties of evoked IPSCs (eIPSCs) were explored using a paired pulse ratio (PPR) stimulus protocol. Electrode stimulus was applied at an interval of 1 s at an intensity of 50% maximum current stimulus. $GABA_A$-mediated currents were isolated in the presence of 50 μM d-APV and 10 μM NBQX. The ratio of the maximum amplitude of the first peak to the maximum amplitude of the second was calculated and compared. The time course of eIPCS decay was described using a single exponential function. AMPA-mediated evoked EPSCs (eEPSCs) were recorded using the same PPR protocol with a shorter 150 ms interval, at a holding potential of −70 mV, in the presence of 20 μM picrotoxin and 50 μM d-APV. NMDA-mediated eEPSCs were recorded using the PPR same protocol with a 500 ms interval at a holding potential of −30 mV, in the presence of 20 μM picrotoxin and 10 μM NBQX. The threshold current stimulus, 50% maximum, and stimulus for maximum EPSC current amplitude were determined. All excitatory synaptic currents were eliminated if 10 μM NBQX and 50 μM d-APV were present in the external solution.

Drug applications. Control currents (pre-drug baseline) and currents after application of drugs were assessed in the same cells. For the acute experiments, all drugs were applied during the time period ranging from 10-15 min until an apparent steady-state effect was achieved. For population study with longer drug applications, 3β-OH and allopregnanolone (Allo) were pre-incubated with brain slices for 60 min before recordings of sIPSCs. The quantitative assessment of drug effects in the intact brain slices is limited since delivery of drug-containing solutions *in vitro* may be compromised due to diffusion through the sliced tissue. Therefore, the actual concentrations of all compounds at their sites of action are likely to be lower than those reported.

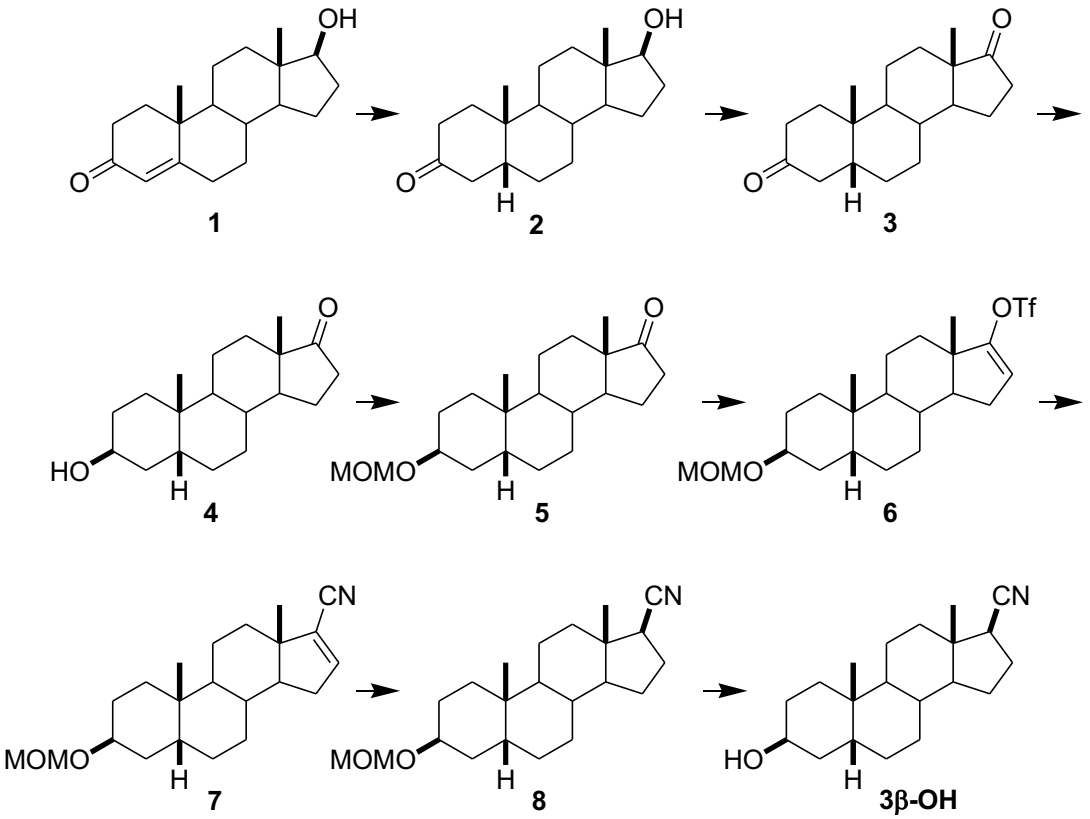

Figure S3: Preparation of (3β,5β,17β)-3-Hydroxyandrostane-17-carbonitrile (3β-OH).

(5β,17β)-17-Hydroxyandrostan-3-one (2).

Testosterone (**1**, 5.2 g, 18 mmol, purchased from Steraloids, Newport, RI, USA), KOH (85%, 1 g, 15 mmol dissolved in 2-propanol, 100 mL), 5% palladium on charcoal (300 mg) and 2-propanol (200 mL) were placed in a Parr hydrogenation flask (500 mL) and hydrogenated at 60 psi for 3 h. Acetic acid (2 mL) was added to the solution to make the solution slightly acidic and the contents of the flask were passed through a silica gel column (flash column grade, 32–63 μm, 500 g). The column was eluted with ethyl acetate and washed with ethyl acetate. The collected solvents were removed under reduced pressure. The resultant solution was made basic by adding aqueous saturated $NaHCO_3$

solution and the contents were diluted with water (300 mL) and extracted with ethyl acetate (150 mL x 3). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4 and the solvent removed to give a pale-yellow oil which was purified by flash column chromatography (silica gel, 32–63 μm eluted with 25-40% ethyl acetate in hexanes) to obtain steroid **2** (3.8 g, 73%).

(5β)-Androstane-3,17-dione (3).

To a solution of steroid **2** (3.8 g, 13.1 mmol) in acetone (150 mL) was added Jones reagent dropwise over a period of 20 min until the orange colour of excess reagent persisted. A few drops of 2-propanol were added to consume the excess reagent and the acetone was removed under reduced pressure. The resulting solution was diluted with water (250 mL) and extracted with ethyl acetate (100 ml x 4). The combined organic extracts were washed with brine, dried over Na_2SO_4 and the solvent removed to give steroid **3** as an oil. Benzene (~200 mL) was added and the benzene was removed on a rotary evaporator to remove traces of water in the product. The resulting product was dried under vacuum for 24 h to yield steroid **3** as white solid (3.7g, 99%).

(3β,5β)-3-Hydroxyandrostan-17-one (4).

To a cold (–78 °C) tetrahydrofuran (200 mL) solution of steroid **3** (3.7 g, 12.9 mmol) was added a 1M tetrahydrofuran solution of K-selectride (*sec*-butylborohydride, 15 mL, 15 mmol, 1.16 eq.) dropwise using a syringe over a period of 15 min and the reaction was stirred at that temperature for another 2 h. The excess reagent was quenched at –78°C by adding few drops of water and the reaction was warmed to room temperature. A 35%

hydrogen peroxide solution (20 mL) and 5 M aqueous NaOH solution (20 mL) were added to the reaction and stirring was continued for 2 h. Water (200 mL) was added and the product extracted with ethyl acetate (125 mL x 4). The combined organic extracts were washed with brine, dried and solvents removed to give an oil which was purified by flash column chromatography (silica gel, 32–63 μm eluted with 25-35% ethyl acetate in hexanes) to give steroid **4** (3.1 g, 83%).

(3β,5β)-3-(Methoxymethoxy)-androstan-17-one (5).

To a cold (0 °C) mixture of steroid **4** (3.1 g, 10.7 mmol), diisopropyl ethylamine (7.45 mL, 42.8 mmol) and dichloromethane (60 mL) was added methoxymethyl chloride (1.63 mL, 21.4 mmol) and the reaction was warmed to room temperature and stirred for 16 h. The reaction was quenched with aqueous saturated $NaHCO_3$ (20 mL) and water was added (100 mL). The biphasic mixture was transferred to separatory funnel and the methylene chloride layer was separated. The aqueous layer was extracted with methylene chloride (3 x 75 mL) and the combined methylene chloride extracts were dried and concentrated to give an oil which was purified by flash column chromatography (silica gel, 32–63 μm eluted with 10-15% ethyl acetate in hexanes) to yield steroid **5** as a white solid (3.3 g, 92%).

(3β,5β)-3-(Methoxymethoxy)-androst-16-en-17-ol, 17-(1,1,1-trifluoromethanesulfonate) (6).

To a cold (–78°C) THF solution of steroid **5** (3.3 g, 9.9 mmol)) and *N*-phenyl bis(trifluoromethanesulfonimide) (4.3 g, 12 mmol)) was added a 0.5 M toluene solution of

potassium hexamethyldisilazide (22 mL, 11 mmol) and the reaction was slowly warmed to room temperature and allowed to stir for 5 h. The reaction was quenched with water and extracted with ethyl acetate (100 mL x 3). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4 and the solvents removed to give a yellow oil which was purified by flash column chromatography (silica gel, 32–63 μm eluted with 2-3% ethyl acetate in hexanes) give steroid **6** as a colorless oil (4.61 g, 99%).

(3β,5β)-3-(Methoxymethoxy)-androst-16-en-17-carbonitrile (7).

An acetonitrile solution (150 mL) of steroid **6** (4.61 g, 9.9 mmol), Cu(I)I (100 mg) and NaCN (980 mg, 20 mmol) was refluxed under a nitrogen atmosphere. To the refluxing mixture was added tertakis(triphenylphosphine)palladium (350 mg, 0.3 mmol) and the reaction was continued at reflux for another 2 hr. The reaction was cooled and aqueous saturated $NaHCO_3$ (50 mL) and water (150 mL) were added. The biphasic solution was transferred to a separatory funnel and extracted with ethyl acetate (100 mL x 4). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4 and the solvents removed to give a crude product which was purified by flash column chromatography (silica gel, 32–63 μm eluted with 5-15% ethyl acetate in hexanes) to give steroid **7** as a white solid. (2.8 g, 82 %).

(3β,5β)-3-(Methoxymethoxy)-androstane-17-carbonitrile (8).

A mixture of steroid **7** (2.8 g, 8.15 mmol), 10% palladium on charcoal (300 mg) and ethyl acetate (200 mL) was placed in a Parr hydrogenation flask and hydrogenated at 60 psi for 7 h. The contents of the flask were passed through a silica gel column (flash column

grade, 32–63 μm, 200 g) and eluted with ethyl acetate. The solvents were removed under reduced pressure and the resulting crude product was purified by flash column chromatography (silica gel, 32–63 μm eluted with 10-15% ethyl acetate in hexanes) to obtain steroid **8** (2.7 g, 96%).

(3β,5β)-3-Hydroxy-androstane-17-carbonitrile (3β-OH).

To a methanolic solution (60 mL) of steroid **8** (2.7 g, 7.8 mmol) was added a dry methanolic HCl solution (15 mL, ~ 5 N prepared in situ by adding acetyl choride to methanol) and the reaction was stirred at room temperature for 4 hr. The reaction was made basic by adding aqueous saturated $NaHCO_3$ and extracted with methylene chloride (80 mL x 4). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvents removed to give crude product **3β-OH** as an off-white solid which was purified by column chromatography (silica gel, 32–63 μm eluted with 25-40% ethyl acetate in hexanes) to give a white solid (2.2 g, 94%). The physical and spectroscopic data were identical to those reported earlier for **3β-OH** prepared by a different procedure (45).

	15% cyclodextrin	saline	3β-OH	ketamine
SpO_2 (%)	[91.2 (2.9)]; n = 4	[93.6 (2.0)]; n = 5	[94.8 (1.2)]; n = 5	[90.6 (2.4)]; n = 5

Supplemental Table 1: The pulse oximeter saturation (SpO_2) levels measured on room air after the fifth and sixth i.p. injections of either 15% cyclodextrin, saline, 3β-OH or ketamine.

	$T_{1/2}$	T_{max}	C_{max}	AUC_{last}	AUC_{inf}
Plasma	8.17 min	5.00 min	1671.40 ng mL^{-1}	25088.00 min · ng mL^{-1}	25275.00 min · ng mL^{-1}
Brain	29.15 min	15.00 min	624.50 ng g^{-1}	24919.00 min · ng g^{-1}	28320.00 min · ng g^{-1}

Supplemental Table 2: Plasma and brain homogenate pharmacokinetic parameters. 3β-OH was quantified using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) in rat EDTA plasma and brain tissues. The time-concentration data was analyzed using a non-compartmental pharmacokinetics model (WinNonlin Phoenix, version 7.0, Certara, Princeton, NJ). 3β-OH was readily distributed into the brain and reached maximum concentrations of 625 ng g^{-1} after 15 min. With 29.2 min, the half-life in brain was 3.6-fold longer than in plasma. Abbreviations: $t_{1/2}$: half-life, t_{max}: time-to-maximum concentration, C_{max}: maximum concentrations, $AUC_{0-\tau}$: area-under-the-concentration-time curve from drug administration to the end of the observation period, $AUC_{0-\infty}$: area-under-the-concentration-time curve from drug administration extrapolated to infinity.

References

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