Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Psychopharmacology (Berl). Author manuscript; available in PMC 2015 December 28.

Published in final edited form as:

Psychopharmacology (Berl). 2014 September ; 231(17): 3415–3423. doi:10.1007/s00213-014-3600-8.

Effects of the Neuroactive Steroid Allopregnanolone on Intracranial Self-Stimulation in C57BL/6J Mice

Eric W. Fish[1], **Buddy J. Whitman**[1], **Jeff F. DiBerto**[2], **J. Elliott Robinson**[2], **A. Leslie Morrow**[#1,3], and **C.J. Malanga**[#1,2]

[1] Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[2]Department of Neurology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[3]Departments of Psychiatry and Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

[#] These authors contributed equally to this work.

Abstract

Rationale—The neuroactive steroid (3α,5α)-3-hydroxy-pregnan-20-one (3α,5α-THP, allopregnanolone) has effects on reward-related behaviors in mice and rats that suggest that it may activate brain reward circuits. Intracranial self-stimulation (ICSS) is an operant behavioral technique that detects changes in the sensitivity of brain reward circuitry following drug administration.

Objective—to examine the effects of the neuroactive steroid allopregnanolone on ICSS and to compare these effects to those of cocaine.

Methods—Male C57BL/6J mice implanted with stimulating electrodes implanted into the medial forebrain bundle responded for reinforcement by electrical stimulation (brain stimulation reward, BSR). Mice received cocaine (n=11, 3.0 – 30.0 mg/kg, i.p.) or the neuroactive steroid allopregnanolone (n=11, 3.0 – 17.0 mg/kg, i.p.). BSR thresholds (θ_0) and maximum operant response rates (MAX) after drug treatments were compared to those after vehicle injections.

Results—Cocaine and allopregnanolone dose dependently lowered BSR thresholds relative to vehicle injections. Cocaine was maximally effective (80 % reduction) in the second 15 minutes following the 30 mg/kg dose, while allopregnanolone was maximally effective (30% reduction) 15-45 minutes after the 17 mg/kg dose. Neither drug had significant effects on MAX response rates.

Conclusions—The effects of allopregnanolone on BSR thresholds are consistent with the previously reported effects of benzodiazepines and alcohol suggesting that positive modulation of $GABA_A$ receptors can facilitate reward-related behaviors in C57BL/6J mice.

Correspondence: Eric W Fish Bowles Center for Alcohol Studies, University of North Carolina at Chapel Hill, CB# 7178, 104 Manning Dr., Chapel Hill, NC 27599.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Keywords

Reward; Reinforcement; Electrical Brain Stimulation; $GABA_A$ Receptors; Dopamine; Psychostimulant

Introduction

Since its discovery as a potent allosteric modulator of $GABA_A$ receptors, the endogenous neuroactive steroid (3α,5α)-3-hydroxy-pregnan-20-one (3α,5α-THP, allopregnanolone has been shown to affect a wide array of behaviors in species as diverse as salamanders and human beings. Roles for endogenously synthesized allopregnanolone have been proposed in reproductive and aggressive behaviors, learning and memory, mood and anxiety, and drug use and withdrawal, while dysregulation of allopregnanolone has been associated with psychiatric conditions such as pre-menstrual dysphoria, depression, and schizophrenia (Brinton 2013; Marx et al. 2006; Rasmusson et al. 2006; Rupprecht et al. 2010). Though the effects of allopregnanolone likely involve potentiation or direct activation of $GABA_A$ receptors (see (Morrow 2007) for review), which are associated with GABA mediated Cl^- influx and cellular inhibition, allopregnanolone alters many behaviors in ways that are similar to those of drugs that enhance the mesolimbic dopamine (DA) system. Allopregnanolone can stimulate locomotor activity in mice (Finn et al. 1997b; Palmer et al. 2002), enhance sexual receptivity and motivation in female rats and hamsters (Frye et al. 1998; Frye and DeBold 1993), facilitate responding for different reinforcers in both mice and rats (Fish et al. 2002; Janak et al. 1998; Sinnott et al. 2002b), increase or decrease mouse aggressive behavior in particular testing conditions (Fish et al. 2001; Pinna et al. 2003), and induce conditioned place preference in mice (Finn et al. 1997a). Certain drugs of abuse, including alcohol, can elevate allopregnanolone levels in the brain as well as the bloodstream, effects that are most consistently observed in rats (Concas et al. 2000; Cook et al. 2014a; Grobin et al. 2005; Morrow et al. 1999; Quinones-Jenab et al. 2008) and allopregnanolone appears to contribute some of the behavioral effects of alcohol (VanDoren et al. 2000). Interestingly, intracerebroventricular (icv) allopregnanolone administration biphasically alters extracellular DA levels in the mesocorticolimbic circuitry (Rouge-Pont et al. 2002) and allopregnanolone is thought to be the mechanism through which progesterone sensitizes DA elevations induced by alcohol (Dazzi et al. 2002).

A common effect of drugs that directly and/or indirectly enhance dopaminergic neurotransmission is to increase operant intracranial self-stimulation (ICSS). ICSS measures the responding of an animal for reinforcement by direct electrical stimulation of brain reward circuitry. Stimulation of the medial forebrain bundle, which contains ascending dopaminergic projections from the ventral tegmental area, as well as descending glutamatergic and GABAergic projections from the cortex and nucleus accumbens (NAc), elicits robust and reproducible brain stimulation reward (BSR). Drugs of abuse with widely differing pharmacological mechanisms of action all potentiate BSR (Kornetsky and Bain 1992). An essential role for DA in BSR is supported by pharmacological studies administering indirect agonists, specific DA receptor agonists and antagonists, as well as *in vivo* measures of extracellular DA levels (Carlezon and Chartoff 2007; Wise 1996).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

However, the full expression of ICSS requires not only DA, but also the integrated functions of glutamate and GABA (Cheer et al. 2005) as well as cholinergic actions in the VTA (Wise 2002). While the glutamatergic (Herberg and Rose 1990; Todtenkopf et al. 2006; You et al. 2001) and cholinergic, especially in regard to nicotine (Huston-Lyons and Kornetsky 1992; Kenny and Markou 2006; Singh et al. 1997), contribution to ICSS has been well characterized, the role of GABA in ICSS has been studied less frequently. Pharmacological studies have demonstrated a potentiation of ICSS by benzodiazepines (Caudarella et al. 1982; Reynolds et al. 2012; Straub et al. 2010) and alcohol (Fish et al. 2010; Robinson et al. 2013) consistent with abuse of these substances by humans. However, the role of GABA in reward processing is complex and other studies have shown that direct GABA agonists depress ICSS (Hayes et al. 2011; Willick and Kokkinidis 1995). Given that allopregnanolone can alter extracellular DA levels in mesocorticolimbic regions and produces behavioral and neurophysiological effects that are similar to benzodiazepines and alcohol, we hypothesized that allopregnanolone could also potentiate ICSS. The present study examined the effects of allopregnanolone on ICSS in male mice and compared these effects to those of the pharmacological reference compound cocaine.

Methods

Mice

The cocaine and allopregnanolone dose-response experiments were conducted in separate groups of experimentally naïve male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME; n=11 for each group). The effects of allopregnanolone were further replicated in an additional replicate of mice with a different experimental history. This replicate consisted of 11 male C57 mice with a humanized mutation of the mu opioid receptor (h/mOPRM1 118AA (n=5) or 118GG (n=6), (Ramchandani et al. 2011) that had previously been treated with the mu opioid receptor agonist fentanyl and the kappa opioid receptor agonist U69,593 (n=8) or the opioid receptor agonist morphine and cocaine (n=3) (Robinson et al. in prep). When the mice were at least 60 days old, monopolar stainless steel electrodes (0.28 mm diameter, Plastics One, Roanoke, VA) were stereotaxically implanted to the right medial forebrain bundle at the level of the lateral hypothalamus (LH) (AP: −1.2; ML −1.0; DV −5.2 from the skull,) under anesthesia with ketamine (120 mg/kg) and xylazine (9 mg/kg) (Sigma, St. Louis MO). The electrode connected to a stainless steel electrical ground screw and was mounted to the skull with dental cement. Following implantation, the mice were housed individually in polycarbonate cages (28 × 17 × 14 cm) that were lined with cob bedding that was changed weekly and covered with stainless steel wire lids. Mice had free access to food (TestDiet) and tap water and were between. The vivarium was 21±1°C, 30-40% humidity, and on 12-h dark/light cycle (lights off at 8:00 AM). All procedures were performed during the dark phase and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of North Carolina and conducted according to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 2011).

Apparatus and Procedures

Testing occurred in sound attenuated operant conditioning chambers as previously described (Fish et al. 2010; Malanga et al. 2008; Robinson et al. 2011). Computers running control

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

software (MED-PC for Windows, version 4.1; Med Associates; St. Albans, VT) recorded wheel spin responses (1/4 turn), activated house lights, and issued stimulation to the electrodes through a swivel commutator and insulated wire (Plastics One, Roanoke, VA). Each response was reinforced by a brief (500 ms) unipolar cathodal square-wave current at a frequency of 158 Hz (pulse width = 100 μs) accompanied by illumination of the house light (500 ms). Responses during the stimulation period were recorded but did not earn additional stimulation. Current intensity was adjusted for each individual mouse and held constant throughout the experiment to maintain at least 40 responses/min (−40 to −150 μA). Rate-frequency curves were generated as the mice responded for a series of decreasing ($0.05\log_{10}$) stimulation frequencies. Stimulation at each frequency was available *ad libitum* for 50 s initiated by a 10-s phase during which 5 non-contingent ("priming") stimulations were presented. During conditioning, each series of 15 frequencies was presented four times (60-min session) and the range of frequencies (therefore, total charge delivered) was adjusted so that the mice only responded during the 3-5 highest frequencies and did not respond during the remaining 12-10 lowest frequencies. When BSR thresholds varied less than 10% on three consecutive days, the mice were habituated to injections and drug testing phases began. The mice were approximately 90 days old at the start of drug testing. Following a 45-min pre-injection baseline (i.e. three series of 15 descending frequencies), the mice were removed from the chamber, injected with either cocaine (saline, 3, 10 and 30 mg/kg, i.p.) or allopregnanolone (BCD vehicle, 3, 5.6, 10, and 17 mg/kg, i.p.) and returned immediately to the operant chambers for 60 minutes during which four series of 15 descending stimulation frequencies were presented. Each drug dose was administered in a random order and separated by at least 48 hours.

Drugs

Cocaine hydrochloride (Sigma, St. Louis, MO) was dissolved in 0.9% saline and allopregnanolone [(3α,5α)-3-hydroxy-pregnan-20-one, purchased from Robert H. Purdy] was suspended with sonication in a 20% (w/v) hydroxypropyl-β-cyclodextrin (Acros Organics, Fairlawn, NJ) solution. For cocaine, doses were calculated as the free base. All drugs were injected intraperitoneally in a volume of 1ml/100 g body weight.

Histology

At the end of the ICSS experiment, the mice were deeply anesthetized with sodium pentobarbital (120 mg/kg) and intracardially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1M PBS. The brains were removed and sectioned (50 μm) on a sliding microtome and stained with cresyl violet for Nissl to determine the location of the most ventral electrode tip placements under low-powered (4-x) light microscopy (Figure 1).

Data Analysis

Customized software analyzed each rate frequency curve to determine the maximum response rate (MAX) and to derive, from least squares regression, the EF50 (the frequency that maintained 50% of maximum responding) and the BSR threshold θ_0 (the x-intercept, or minimum frequency that maintained ICSS responding). For each mouse, data following drug or vehicle injections were then normalized to a percent of that individual's vehicle baseline.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

All data were analyzed using a one-way repeated measures analysis of variance (ANOVA) with cocaine or allopregnanolone dose as the within subjects factor. Significant F-tests were further analyzed using Bonferroni corrected *post-hoc* tests to determine doses that were significantly different from the vehicle control at the $p<0.05$ level.

Results

The ventral most electrode placements are shown in Figure 1 throughout the right medial forebrain bundle in the lateral hypothalamus. There was a similar distribution of electrode placements and similar pre-injection baseline responding for the mice treated with cocaine and the mice treated with allopregnanolone; BSR thresholds, EF50s and MAX response rates were 2.3±0.2 Coulombs 10^{-7}, 3.2±0.25 Coulombs 10^{-7}, and 143±8.8 responses/50s for the cocaine treated mice and 3.2±0.4 Coulombs 10^{-7}, 4.3±0.39 Coulombs 10^{-7}, and 133±7.4 responses/50s for the allopregnanolone treated mice.

Cocaine

Cocaine dose dependently lowered BSR threshold in each 15-minute time period after injection (Figure2A. $F_{3,43}$= 27.9; 48.9; 33.2; and 8.2 for the first, second, third, and fourth 15 minutes; all p's <0.001). Cocaine also lowered the EF50 (Table 1. $F_{3,43}$= 9.9, 36.3, 19.4, and 6.5 for the first, second, third, and fourth 15 minutes; all p's <0.001). Post-hoc comparisons versus the saline control revealed that the 3 mg/kg dose lowered the EF50 by 18±3.8% in the first 15 minutes, that the 10 mg/kg dose significantly lowered BSR threshold by 50±7.2% and 11±3.9% in the first and second 15 minutes and lowered the EF50 by 30±4.1% and 20±2.6% in the first and second 15 minutes, and that the 30 mg/kg dose significantly lowered BSR threshold by 75±5.3%, 85±2.6%, 72±6.0, and 48±7.6 in the first, second, third, and fourth 15-minute time period. The 30 mg/kg dose also lowered the EF50 by 37±6.6%, 50±3.8%, 46±4.3%, and 32±6.3% in the first, second, third, and fourth 15-minute time period.in each 15-minute period. No dose of cocaine significantly affected MAX response rates as compared to the saline control (Figure 2B).

Allopregnanolone

Allopregnanolone dose-dependently lowered BSR threshold and the EF50 in the second ($F_{4,54}$=4.1; p=0.01, Figure 3A; $F_{4,54}$=3.3; p=0.02, Table 1) and third ($F_{4,54}$=3.0; p=0.03, Figure 3A; $F_{4,54}$=3.9; p=0.01, Table 1) 15-minute time periods. Relative to the vehicle injection, the 17 mg/kg dose lowered BSR threshold and the EF50 by 33±9.0% and 15±3.4%, respectively, in the second 15-minute time period. In the third 15-minute time period, the 17 mg/kg dose lowered BSR threshold and the EF50 by 23±5.6 and 18±5.1%, respectively. There were no significant effects of allopregnanolone on BSR threshold or the EF50 in the first or fourth 15-minute time period. There were no significant effects of allopregnanolone on MAX response rates in any of the 15-minute time periods.

Similar effects of allopregnanolone were achieved when the allopregnanolone was further tested in an additional replicate of mice (Table 2). In this second replicate, the 10 mg/kg dose significantly lowered BSR threshold in the second 15-minute time period and the EF50 in the third 15-minute time period. The 17 mg/kg dose significantly lowered BSR threshold

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

in the third 15-minute time period. There were not significant effects of allopregnanolone on MAX response rates.

Discussion

These experiments compared the effects of different doses of the neuroactive steroid allopregnanolone on ICSS, a behavioral measure of the sensitivity to rewarding brain stimulation. The psychomotor stimulant cocaine was also tested as a pharmacological reference. Allopregnanolone, like cocaine, dose-dependently potentiated ICSS in male C57 mice by lowering the amount of stimulation that was necessary to maintain ICSS responding, as measured by both the EF50 and the θ_0, two indices of the self-stimulation threshold. These effects were significant for the 17 mg/kg dose between 16 and 45 minutes after allopregnanolone injection. A possible effect of allopregnanolone on operant motor behavior was not evident as there were no significant increases in maximum (MAX) response rate after any allopregnanolone dose. Overall, these results are consistent with previous findings that other $GABA_A$ receptor positive allosteric modulators, such as benzodiazepines and alcohol can potentiate BSR (Fish et al. 2010; Reynolds et al. 2012; Straub et al. 2010).

Allopregnanolone was tested in two separate replicates of mice, one which had a prior drug history and one that was experimentally naïve. Two important features of ICSS are that BSR thresholds remain stable over time (Carlezon and Chartoff 2007; Riday et al. 2012b) and the sensitivity to pharmacological manipulation does not change after prior experience with psychostimulants or opioids (Esposito and Kornetsky 1977; Frank et al. 1988; Riday et al. 2012b). The dose-dependent effects of allopregnanolone on ICSS were qualitatively similar in each of the replicates, supporting the validity of testing different pharmacological classes of drugs in the same individuals (Fish et al. 2013; Malanga et al. 2008; Riday et al. 2012a) and strengthening the conclusion that allopregnanolone potentiates BSR in C57BL/6J mice. Whether repeated allopregnanolone treatment induces tolerance and/or sensitization to its effects on BSR is a question for further study. Additionally, there are species and strain differences in response to allopregnanolone and it is not known how these results would generalize to rats or to different mouse strains (Finn et al. 1997b; Porcu et al. 2010) (see Porcu et al., this issue)

The largest effects of allopregnanolone on ICSS in C57BL/6J mice occurred after the 10 and 17 mg/kg doses, a dose range that is consistent with other behavioral effects of allopregnanolone, such as locomotor stimulation (Finn et al. 1997b; Palmer et al. 2002), escalated aggression (Fish et al. 2001), reduction of anxiety-like behaviors (Bitran et al. 1991; Brot et al. 1997; Fish et al. 2000) and alcohol consumption (Ford et al. 2005). The 17 mg/kg dose allopregnanolone significantly lowered self-stimulation thresholds by about 30%. This degree of reduction was of a similar magnitude to the effects observed immediately after 3 mg/kg cocaine and was modest compared to the maximal effects of the 30 mg/kg dose of cocaine. Moderate reductions in BSR thresholds have been reported for the benzodiazepines diazepam (Reynolds et al. 2012) and midazolam (Engin et al. 2014) as well as for alcohol (Fish et al. 2010), indicating that $GABA_A$ receptor positive modulators can potentiate BSR, although not to the same degree as psychostimulants. The time course

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

for allopregnanolone followed previously reported brain levels of allopregnanolone after systemic administration which peak within 10 minutes and are metabolized rapidly into forms that do not affect $GABA_A$ receptors (Mellon et al. 2008; Purdy et al. 1990). An alternative to allopregnanolone treatment is the synthetic neurosteroid ganaxolone, which has a longer half-life (Carter et al. 1997) and may be useful for the treatment of epilepsy (Reddy 2010). Future measurements of the effects of ganaxolone on ICSS may uncover influences on brain reward that could be related to its effects on alcohol intake in rodents (Besheer et al. 2010; Ramaker et al. 2012) and have implications for its clinical use.

The potentiation of BSR is consistent with the findings that lower doses of allopregnanolone and ganaxolone can facilitate some other operant behaviors, including fixed interval responding for the opportunity for aggression (Fish et al. 2002), fixed ratio responding for alcohol administration (Besheer et al. 2010; Janak et al. 1998; Ramaker et al. 2012) and the reinstatement of extinguished responding for alcohol (Finn et al. 2008). Some evidence also suggests allopregnanolone has reinforcing effects of its own as mice will orally self-administer allopregnanolone (Sinnott et al. 2002a) and develop a conditioned place preference (Finn et al. 1997a), but see Beauchamp (2000). The current results are not interpreted to reflect a direct reinforcing effect of allopregnanolone. Instead, allopregnanolone, like many drugs that are self-administered, enhances sensitivity to BSR. This enhanced sensitivity combined with the above mentioned increased responding for certain reinforcers suggests that one of the effects of allopregnanolone may be an overall increase in the salience of positive reinforcement. In contrast to this hypothesis are the findings that allopregnanolone, especially at higher doses, inhibits responding in rats reinforced by cocaine or food administration (Anker and Carroll 2010; Schmoutz et al. 2014) as well as the reinstatement of extinguished responding for cocaine (Anker et al. 2009; Schmoutz et al. 2014). Allopregnanolone can attenuate cocaine-induced seizures (Kaminski et al. 2003) and high doses of other neuroactive steroids can decrease the sensitivity to the discriminative stimulus effects of cocaine (Quinton et al. 2006) and alter cocaine-induced conditioned placed preference (Romieu et al. 2003). The findings of these studies indicate that allopregnanolone influences several effects of cocaine and that allopregnanolone may differentially alter specific forms of reward and reinforcement.

An important consideration, which cannot be directly addressed in the current study, is whether allopregnanolone potentiates BSR through a dopamine dependent mechanism, as do many other drugs that potentiate BSR and stimulate locomotor behavior (Wise 2002) or if its actions are independent of dopamine, as has been suggested for the effects of benzodiazepines (Straub et al. 2010). There are numerous interactions between allopregnanolone and DA that suggest that DA could be involved in the effects of allopregnanolone on BSR. For example, in the VTA, allopregnanolone co-localizes with tyrosine hydroxylase, the rate-limiting enzyme in DA biosynthesis (Cook et al. 2014b) where it may modulate local excitability (Akk et al. 2005; Sanna et al. 2004). Ganaxolone and benzodiazepines can act on DA neurons in the VTA and enhance long-term potentiation (Tan et al. 2010; Vashchinkina et al. 2014) an effect associated with altered place conditioning. Moreover, pharmacological antagonists to dopamine receptors attenuate the promotion of lordosis behavior by ventral tegmental area (VTA) allopregnanolone in female rats (Frye et al. 2004).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

While the above evidence supports the idea that dopamine might also be involved in the effects of allopregnanolone on BSR, Straub et al. (2010) hypothesized that the potentiating effects of benzodiazepines on BSR are due to direct inhibition of medium spiny neurons in the NAc, which has been proposed as important for reward processing (Carlezon and Thomas 2009). It is not known whether allopregnanolone modulates these medium spiny neurons but a GABAergic mechanism is thought to inhibit NAc core neurons following MFB stimulation (Cheer et al. 2005). Neurons expressing the $GABA_A$ receptor α2 subunit are critical for the BSR potentiating effects of diazepam (Reynolds et al. 2012) and midazolam (Engin et al. 2014), and extrasynaptic α4 subunit containing $GABA_A$ receptors in the NAc modulate the place conditioning effects of cocaine (Maguire et al. 2014) indicating the ability of specific $GABA_A$ receptors to influence measures of reward. Given the roles of both DA and GABA in the neural control of reward and reinforcement processing and the fact that neuroactive steroids modulate components of both of these neurotransmitters, it is tempting to speculate that allopregnanolone may be an endogenous positive modulator of mesocorticolimbic reward circuits. Continued investigations with the ICSS technique may help to resolve whether the facilitation of BSR by allopregnanolone is predominantly dopaminergic or GABAergic.

Acknowledgement

This work was supported byAA 018335 to CJM, AA016672 to ALM, and T32-AA007573 to the Bowles Center of Alcohol Studies

REFERENCES

Akk G, Shu HJ, Wang C, Steinbach JH, Zorumski CF, Covey DF, Mennerick S. Neurosteroid access to the GABAA receptor. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2005; 25:11605–13. [PubMed: 16354918]

Anker JJ, Carroll ME. The role of progestins in the behavioral effects of cocaine and other drugs of abuse: human and animal research. Neuroscience and biobehavioral reviews. 2010; 35:315–33. [PubMed: 20398693]

Anker JJ, Holtz NA, Zlebnik N, Carroll ME. Effects of allopregnanolone on the reinstatement of cocaine-seeking behavior in male and female rats. Psychopharmacology. 2009; 203:63–72. [PubMed: 18998113]

Beauchamp MH, Ormerod BK, Jhamandas K, Boegman RJ, Beninger RJ. Neurosteroids and reward: allopregnanolone produces a conditioned place aversion in rats. Pharmacology, biochemistry, and behavior. 2000; 67:29–35.

Besheer J, Lindsay TG, O'Buckley TK, Hodge CW, Morrow AL. Pregnenolone and ganaxolone reduce operant ethanol self-administration in alcohol-preferring p rats. Alcoholism, clinical and experimental research. 2010; 34:2044–52.

Bitran D, Hilvers RJ, Kellogg CK. Anxiolytic effects of 3 alpha-hydroxy-5 alpha[beta]-pregnan-20-one: endogenous metabolites of progesterone that are active at the GABAA receptor. Brain research. 1991; 561:157–61. [PubMed: 1686744]

Brinton RD. Neurosteroids as regenerative agents in the brain: therapeutic implications. Nat Rev Endocrinol. 2013; 9:241–50. [PubMed: 23438839]

Brot MD, Akwa Y, Purdy RH, Koob GF, Britton KT. The anxiolytic-like effects of the neurosteroid allopregnanolone: interactions with GABA(A) receptors. European journal of pharmacology. 1997; 325:1–7. [PubMed: 9151931]

Carlezon WA Jr. Chartoff EH. Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. Nat Protoc. 2007; 2:2987–95. [PubMed: 18007634]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Carlezon WA Jr. Thomas MJ. Biological substrates of reward and aversion: a nucleus accumbens activity hypothesis. Neuropharmacology. 2009; 56(Suppl 1):122–32. [PubMed: 18675281]

Carter RB, Wood PL, Wieland S, Hawkinson JE, Belelli D, Lambert JJ, White HS, Wolf HH, Mirsadeghi S, Tahir SH, Bolger MB, Lan NC, Gee KW. Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3alpha-hydroxy-3beta-methyl-5alpha-pregnan-20-one), a selective, high-affinity, steroid modulator of the gamma-aminobutyric acid(A) receptor. The Journal of pharmacology and experimental therapeutics. 1997; 280:1284–95. [PubMed: 9067315]

Caudarella M, Campbell KA, Milgram NW. Differential effects of diazepam (valium) on brain stimulation reward sites. Pharmacology, biochemistry, and behavior. 1982; 16:17–21.

Cheer JF, Heien ML, Garris PA, Carelli RM, Wightman RM. Simultaneous dopamine and single-unit recordings reveal accumbens GABAergic responses: implications for intracranial self-stimulation. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:19150–5. [PubMed: 16380429]

Concas A, Porcu P, Sogliano C, Serra M, Purdy RH, Biggio G. Caffeine-induced increases in the brain and plasma concentrations of neuroactive steroids in the rat. Pharmacology, biochemistry, and behavior. 2000; 66:39–45.

Cook JB, Dumitru AM, O'Buckley TK, Morrow AL. Ethanol administration produces divergent changes in GABAergic neuroactive steroid immunohistochemistry in the rat brain. Alcoholism, clinical and experimental research. 2014a; 38:90–9.

Cook JB, Werner DF, Maldonado-Devincci AM, Leonard MN, Fisher KR, O'Buckley TK, Porcu P, McCown TJ, Besheer J, Hodge CW, Morrow AL. Overexpression of the steroidogenic enzyme cytochrome P450 side chain cleavage in the ventral tegmental area increases 3α,5α-THP and reduces long-term operant ethanol self-administration. J Neurosci. 2014b In press.

Dazzi L, Serra M, Seu E, Cherchi G, Pisu MG, Purdy RH, Biggio G. Progesterone enhances ethanol-induced modulation of mesocortical dopamine neurons: antagonism by finasteride. Journal of neurochemistry. 2002; 83:1103–9. [PubMed: 12437581]

Engin E, Bakhurin KI, Smith KS, Hines RM, Reynolds LM, Tang W, Sprengel R, Moss SJ, Rudolph U. Neural Basis of Benzodiazepine Reward: Requirement for alpha2 Containing GABA Receptors in the Nucleus Accumbens. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2014

Esposito R, Kornetsky C. Morphine lowering of self-stimulation thresholds: lack of tolerance with long-term administration. Science. 1977; 195:189–91. [PubMed: 831268]

Finn DA, Mark GP, Fretwell AM, Gililland-Kaufman KR, Strong MN, Ford MM. Reinstatement of ethanol and sucrose seeking by the neurosteroid allopregnanolone in C57BL/6 mice. Psychopharmacology. 2008; 201:423–33. [PubMed: 18758755]

Finn DA, Phillips TJ, Okorn DM, Chester JA, Cunningham CL. Rewarding effect of the neuroactive steroid 3 alpha-hydroxy-5 alpha-pregnan-20-one in mice. Pharmacology, biochemistry, and behavior. 1997a; 56:261–4.

Finn DA, Roberts AJ, Lotrich F, Gallaher EJ. Genetic differences in behavioral sensitivity to a neuroactive steroid. The Journal of pharmacology and experimental therapeutics. 1997b; 280:820–8. [PubMed: 9023296]

Fish EW, De Bold JF, Miczek KA. Aggressive behavior as a reinforcer in mice: activation by allopregnanolone. Psychopharmacology. 2002; 163:459–66. [PubMed: 12373446]

Fish EW, Faccidomo S, DeBold JF, Miczek KA. Alcohol, allopregnanolone and aggression in mice. Psychopharmacology. 2001; 153:473–83. [PubMed: 11243495]

Fish EW, Krouse MC, Stringfield SJ, Diberto JF, Robinson JE, Malanga CJ. Changes in sensitivity of reward and motor behavior to dopaminergic, glutamatergic, and cholinergic drugs in a mouse model of fragile x syndrome. PLoS One. 2013; 8:e77896. [PubMed: 24205018]

Fish EW, Riday TT, McGuigan MM, Faccidomo S, Hodge CW, Malanga CJ. Alcohol, cocaine, and brain stimulation-reward in C57Bl6/J and DBA2/J mice. Alcohol Clin Exp Res. 2010; 34:81–9. [PubMed: 19860803]

Fish EW, Sekinda M, Ferrari PF, Dirks A, Miczek KA. Distress vocalizations in maternally separated mouse pups: modulation via 5-HT(1A), 5-HT(1B) and GABA(A) receptors. Psychopharmacology. 2000; 149:277–85. [PubMed: 10823409]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Ford MM, Nickel JD, Phillips TJ, Finn DA. Neurosteroid modulators of GABA(A) receptors differentially modulate Ethanol intake patterns in male C57BL/6J mice. Alcoholism, clinical and experimental research. 2005; 29:1630–40.

Frank RA, Martz S, Pommering T. The effect of chronic cocaine on self-stimulation train-duration thresholds. Pharmacology, biochemistry, and behavior. 1988; 29:755–8.

Frye CA, Bayon LE, Pursnani NK, Purdy RH. The neurosteroids, progesterone and 3alpha,5alpha-THP, enhance sexual motivation, receptivity, and proceptivity in female rats. Brain research. 1998; 808:72–83. [PubMed: 9795145]

Frye CA, DeBold JF. 3 alpha-OH-DHP and 5 alpha-THDOC implants to the ventral tegmental area facilitate sexual receptivity in hamsters after progesterone priming to the ventral medial hypothalamus. Brain research. 1993; 612:130–7. [PubMed: 8330193]

Frye CA, Walf AA, Sumida K. Progestins' actions in the VTA to facilitate lordosis involve dopamine-like type 1 and 2 receptors. Pharmacology, biochemistry, and behavior. 2004; 78:405–18.

Grobin AC, VanDoren MJ, Porrino LJ, Morrow AL. Cortical 3 alpha-hydroxy-5 alpha-pregnan-20-one levels after acute administration of Delta 9-tetrahydrocannabinol, cocaine and morphine. Psychopharmacology. 2005; 179:544–50. [PubMed: 15619118]

Hayes DJ, Hoang J, Greenshaw AJ. The role of nucleus accumbens shell GABA receptors on ventral tegmental area intracranial self-stimulation and a potential role for the 5-HT(2C) receptor. J Psychopharmacol. 2011; 25:1661–75. [PubMed: 21169393]

Herberg LJ, Rose IC. Excitatory amino acid pathways in brain-stimulation reward. Behavioural brain research. 1990; 39:230–9. [PubMed: 1978779]

Huston-Lyons D, Kornetsky C. Effects of nicotine on the threshold for rewarding brain stimulation in rats. Pharmacol Biochem Behav. 1992; 41:755–9. [PubMed: 1594644]

Janak PH, Redfern JE, Samson HH. The reinforcing effects of ethanol are altered by the endogenous neurosteroid, allopregnanolone. Alcoholism, clinical and experimental research. 1998; 22:1106–12.

Kaminski RM, Gasior M, Carter RB, Witkin JM. Protective efficacy of neuroactive steroids against cocaine kindled-seizures in mice. European journal of pharmacology. 2003; 474:217–22. [PubMed: 12921865]

Kenny PJ, Markou A. Nicotine self-administration acutely activates brain reward systems and induces a long-lasting increase in reward sensitivity. Neuropsychopharmacology. 2006; 31:1203–11. [PubMed: 16192981]

Kornetsky C, Bain G. Brain-stimulation reward: a model for the study of the rewarding effects of abused drugs. NIDA Res Monogr. 1992; 124:73–93. [PubMed: 1470216]

Maguire EP, Macpherson T, Swinny JD, Dixon CI, Herd MB, Belelli D, Stephens DN, King SL, Lambert JJ. Tonic inhibition of accumbal spiny neurons by extrasynaptic alpha4betadelta GABAA receptors modulates the actions of psychostimulants. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2014; 34:823–38. [PubMed: 24431441]

Malanga CJ, Riday TT, Carlezon WA Jr. Kosofsky BE. Prenatal exposure to cocaine increases the rewarding potency of cocaine and selective dopaminergic agonists in adult mice. Biol Psychiatry. 2008; 63:214–21. [PubMed: 17568568]

Marx CE, Stevens RD, Shampine LJ, Uzunova V, Trost WT, Butterfield MI, Massing MW, Hamer RM, Morrow AL, Lieberman JA. Neuroactive steroids are altered in schizophrenia and bipolar disorder: relevance to pathophysiology and therapeutics. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2006; 31:1249–63. [PubMed: 16319920]

Mellon SH, Gong W, Schonemann MD. Endogenous and synthetic neurosteroids in treatment of Niemann-Pick Type C disease. Brain Res Rev. 2008; 57:410–20. [PubMed: 17629950]

Morrow AL. Recent developments in the significance and therapeutic relevance of neuroactive steroids--Introduction to the special issue. Pharmacology & therapeutics. 2007; 116:1–6. [PubMed: 17531324]

Morrow AL, Janis GC, VanDoren MJ, Matthews DB, Samson HH, Janak PH, Grant KA. Neurosteroids mediate pharmacological effects of ethanol: a new mechanism of ethanol action? Alcoholism, clinical and experimental research. 1999; 23:1933–40.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Palmer AA, Miller MN, McKinnon CS, Phillips TJ. Sensitivity to the locomotor stimulant effects of ethanol and allopregnanolone is influenced by common genes. Behav Neurosci. 2002; 116:126–37. [PubMed: 11895174]

Pinna G, Dong E, Matsumoto K, Costa E, Guidotti A. In socially isolated mice, the reversal of brain allopregnanolone down-regulation mediates the anti-aggressive action of fluoxetine. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100:2035–40. [PubMed: 12571361]

Porcu P, O'Buckley TK, Alward SE, Song SC, Grant KA, de Wit H, Morrow AL. Differential Effects of Ethanol on Serum GABAergic 3 alpha,5 alpha/3 alpha,5 beta Neuroactive Steroids in Mice, Rats, Cynomolgus Monkeys, and Humans. Alcoholism-Clinical and Experimental Research. 2010; 34:432–442.

Purdy RH, Morrow AL, Blinn JR, Paul SM. Synthesis, metabolism, and pharmacological activity of 3 alpha-hydroxy steroids which potentiate GABA-receptor-mediated chloride ion uptake in rat cerebral cortical synaptoneurosomes. Journal of medicinal chemistry. 1990; 33:1572–81. [PubMed: 2160534]

Quinones-Jenab V, Minerly AC, Niyomchia T, Akahvan A, Jenab S, Frye C. Progesterone and allopregnanolone are induced by cocaine in serum and brain tissues of male and female rats. Pharmacology, biochemistry, and behavior. 2008; 89:292–7.

Quinton MS, Gerak LR, Moerschbaecher JM, Winsauer PJ. Effects of pregnanolone in rats discriminating cocaine. Pharmacology, biochemistry, and behavior. 2006; 85:385–92.

Ramaker MJ, Strong MN, Ford MM, Finn DA. Effect of ganaxolone and THIP on operant and limited-access ethanol self-administration. Neuropharmacology. 2012; 63:555–64. [PubMed: 22613838]

Ramchandani VA, Umhau J, Pavon FJ, Ruiz-Velasco V, Margas W, Sun H, Damadzic R, Eskay R, Schoor M, Thorsell A, Schwandt ML, Sommer WH, George DT, Parsons LH, Herscovitch P, Hommer D, Heilig M. A genetic determinant of the striatal dopamine response to alcohol in men. Mol Psychiatry. 2011; 16:809–17. [PubMed: 20479755]

Rasmusson AM, Pinna G, Paliwal P, Weisman D, Gottschalk C, Charney D, Krystal J, Guidotti A. Decreased cerebrospinal fluid allopregnanolone levels in women with posttraumatic stress disorder. Biological psychiatry. 2006; 60:704–13. [PubMed: 16934764]

Reddy DS. Neurosteroids: endogenous role in the human brain and therapeutic potentials. Progress in brain research. 2010; 186:113–37. [PubMed: 21094889]

Reynolds LM, Engin E, Tantillo G, Lau HM, Muschamp JW, Carlezon WA Jr. Rudolph U. Differential roles of GABA(A) receptor subtypes in benzodiazepine-induced enhancement of brain-stimulation reward. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2012; 37:2531–40. [PubMed: 22763624]

Riday TT, Dankoski EC, Krouse MC, Fish EW, Walsh PL, Han JE, Hodge CW, Wightman RM, Philpot BD, Malanga CJ. Pathway-specific dopaminergic deficits in a mouse model of Angelman syndrome. The Journal of clinical investigation. 2012a; 122:4544–54. [PubMed: 23143301]

Riday TT, Kosofsky BE, Malanga CJ. The rewarding and locomotor-sensitizing effects of repeated cocaine administration are distinct and separable in mice. Neuropharmacology. 2012b; 62:1858–66. [PubMed: 22197517]

Robinson JE, Chen M, Stamatakis AM, Krouse MC, Howard EC, Faccidomo S, Hodge CW, Fish EW, Malanga CJ. Levetiracetam has opposite effects on alcohol- and cocaine-related behaviors in C57BL/6J mice. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2013; 38:1322–33. [PubMed: 23353709]

Robinson JE, Fish EW, Krouse MC, Thorsell A, Heilig M, Malanga CJ. Potentiation of brain stimulation reward by morphine: effects of neurokinin-1 receptor antagonism. Psychopharmacology (Berl). 2011

Romieu P, Martin-Fardon R, Bowen WD, Maurice T. Sigma 1 receptor-related neuroactive steroids modulate cocaine-induced reward. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2003; 23:3572–6. [PubMed: 12736327]

Rouge-Pont F, Mayo W, Marinelli M, Gingras M, Le Moal M, Piazza PV. The neurosteroid allopregnanolone increases dopamine release and dopaminergic response to morphine in the rat

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

nucleus accumbens. The European journal of neuroscience. 2002; 16:169–73. [PubMed: 12153544]

Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, Groyer G, Adams D, Schumacher M. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. Nat Rev Drug Discov. 2010; 9:971–88. [PubMed: 21119734]

Sanna E, Talani G, Busonero F, Pisu MG, Purdy RH, Serra M, Biggio G. Brain steroidogenesis mediates ethanol modulation of GABAA receptor activity in rat hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004; 24:6521–30. [PubMed: 15269263]

Schmoutz CD, Runyon SP, Goeders NE. Effects of inhibitory GABA-active neurosteroids on cocaine seeking and cocaine taking in rats. Psychopharmacology. 2014

Singh J, Desiraju T, Raju TR. Cholinergic and GABAergic modulation of self-stimulation of lateral hypothalamus and ventral tegmentum: effects of carbachol, atropine, bicuculline, and picrotoxin. Physiology & behavior. 1997; 61:411–8. [PubMed: 9089760]

Sinnott RS, Mark GP, Finn DA. Reinforcing effects of the neurosteroid allopregnanolone in rats. Pharmacology, biochemistry, and behavior. 2002a; 72:923–9.

Sinnott RS, Phillips TJ, Finn DA. Alteration of voluntary ethanol and saccharin consumption by the neurosteroid allopregnanolone in mice. Psychopharmacology. 2002b; 162:438–47. [PubMed: 12172699]

Straub CJ, Carlezon WA Jr. Rudolph U. Diazepam and cocaine potentiate brain stimulation reward in C57BL/6J mice. Behavioural brain research. 2010; 206:17–20. [PubMed: 19716385]

Tan KR, Brown M, Labouebe G, Yvon C, Creton C, Fritschy JM, Rudolph U, Luscher C. Neural bases for addictive properties of benzodiazepines. Nature. 2010; 463:769–74. [PubMed: 20148031]

Todtenkopf MS, Parsegian A, Naydenov A, Neve RL, Konradi C, Carlezon WA Jr. Brain reward regulated by AMPA receptor subunits in nucleus accumbens shell. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2006; 26:11665–9. [PubMed: 17093088]

VanDoren MJ, Matthews DB, Janis GC, Grobin AC, Devaud LL, Morrow AL. Neuroactive steroid 3alpha-hydroxy-5alpha-pregnan-20-one modulates electrophysiological and behavioral actions of ethanol. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2000; 20:1982–9. [PubMed: 10684899]

Vashchinkina E, Manner AK, Vekovischeva O, den Hollander B, Uusi-Oukari M, Aitta-Aho T, Korpi ER. Neurosteroid Agonist at GABAA receptor induces persistent neuroplasticity in VTA dopamine neurons. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2014; 39:727–37. [PubMed: 24077066]

Willick ML, Kokkinidis L. The effects of ventral tegmental administration of GABAA, GABAB and NMDA receptor agonists on medial forebrain bundle self-stimulation. Behavioural brain research. 1995; 70:31–6. [PubMed: 8519426]

Wise RA. Addictive drugs and brain stimulation reward. Annu Rev Neurosci. 1996; 19:319–40. [PubMed: 8833446]

Wise RA. Brain reward circuitry: insights from unsensed incentives. Neuron. 2002; 36:229–40. [PubMed: 12383779]

You ZB, Chen YQ, Wise RA. Dopamine and glutamate release in the nucleus accumbens and ventral tegmental area of rat following lateral hypothalamic self-stimulation. Neuroscience. 2001; 107:629–39. [PubMed: 11720786]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

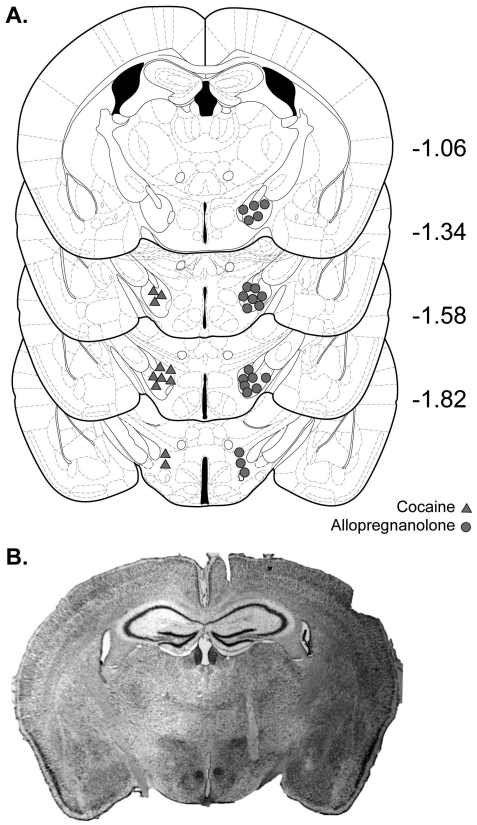

Figure 1.

Panel A. Schematic representation of ICSS electrode implantations aimed for the right medial forebrain bundle at the level of the lateral hypothalamus. For clarity, implantations for the cocaine experiment (n=11, *filled triangles*) are shown on the left and implantations for the allopregnanolone experiments (n=22, *filled circles*) are shown on the right. Panel B. Sample photomicrograph of medial forebrain bundle implantation site.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

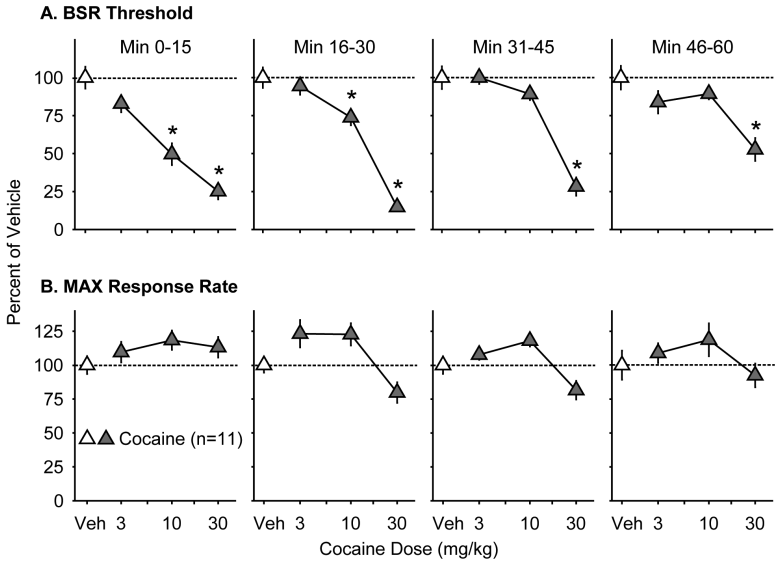

Figure 2.

Time course for the effects of cocaine on ICSS in C57BL/6J mice (n=11). Panel A. (*top*) portrays the effects of cocaine (3, 10, 30 mg/kg, *filled triangles*) or saline (Veh, *open triangles*) on the mean (±SEM) threshold for brain stimulation reward (BSR). Panel B. (*bottom*) portrays the effects of cocaine or saline on the mean (±SEM) maximum response rate (MAX). All data are expressed as a percent of the values for the saline vehicle and are shown over four successive 15 minute intervals. Asterisks denote doses that are significantly different from the saline vehicle ($p<0.05$).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

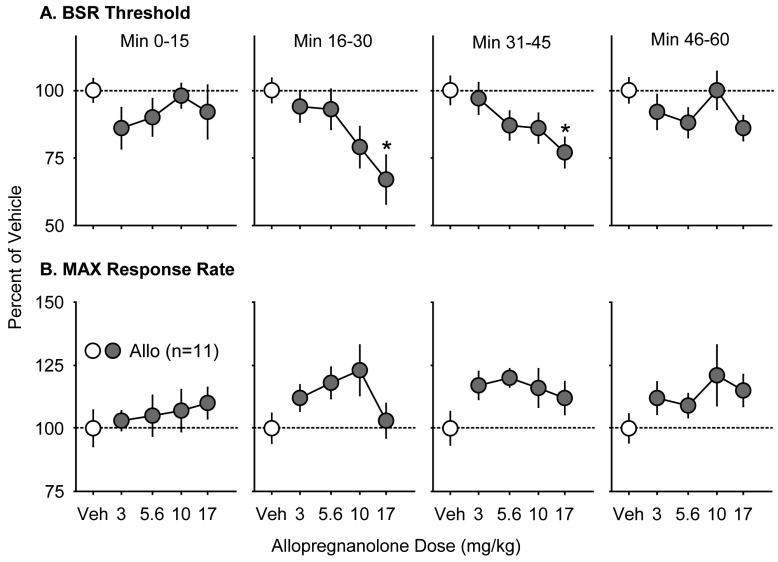

Figure 3.

Time course for the effects of allopregnanolone on ICSS in C57BL/6J mice (n=11). Panel A. (*top*) portrays the effects of allopregnanolone (3, 5.6, 10, 17 mg/kg, *filled circles*) or 20% beta-cyclodextrin (Veh, *open circles*) on the mean (±SEM) threshold for brain stimulation reward (BSR). Panel B. (*bottom*) portrays the effects of allopregnanolone or beta-cyclodextrin on the mean (±SEM) maximum response rate (MAX). All data are expressed as a percent of the values for the saline vehicle and are shown over four successive 15 minute intervals. Asterisks denote doses that are significantly different from the saline vehicle ($p<0.05$).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Effects of Cocaine and Allopregnanolone on the EF_{50} in C57BL/6J Mice.

Time (min)	(0-15)	(16-30)	(31-46)	(46-60)
Cocaine Dose (mg/kg)				
V	100±6.7	100±6.2	100±6.6	100±7.3
3.0	**82±3.8**	93±1.9	95±2.5	97±3.5
10	**70±4.1**	**80±2.6**	85±2.7	90±3.1
30	**63±6.6**	**50±3.8**	**54±4.3**	**68±6.3**
Allopregnanolone Dose (mg/kg)				
V	100±3.4	100±3.1	100±3.5	100±5.2
3.0	100±3.9	98±2.8	98±2.1	98±3.0
5.6	97±2.5	99±3.2	94±3.8	94±3.7
10	99±4.3	98±5.5	93±3.8	100±3.4
17	98±3.5	**85±3.4**	**82±5.1**	90±3.6

All data are mean (±SEM) percent of vehicle baseline. **Emboldened** values are $p<0.05$ vs. vehicle control.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 2

Effects of Allopregnanolone on ICSS Responding in a Second Replicate of C57BL/6J Mice (n=11)

BSR Threshold						
Allo Dose (mg/kg)	*Veh*	*3.0*	*5.6*	*10.0*	*17.0*	*ANOVA*
Time (min)						
0-15	100±6.8	86±7.1	96±9.7	72±4.6	95±14	$F_{(4,54)}$=1.7;p=0.17
16-30	100±6.0	99±8.2	88±9.2	**64±9.1**	76±9.7	$F_{(4,54)}$=3.6;p=0.01
31-45	100±3.5	97±6.8	87±5.5	74±9.9	**70±9.6**	$F_{(4,54)}$=3.7;p=0.01
46-60	100±5.3	99±6.9	94±7.2	81±9.8	82±7.3	$F_{(4,54)}$=1.7;p=0.16
EF50						
Allo Dose (mg/kg)	*Veh*	*3.0*	*5.6*	*10.0*	*17.0*	*ANOVA*
Time (min)						
0-15	100±4.6	98±3.5	100±4.1	85±6.8	100±7.7	$F_{(4,54)}$=1.5;p=0.21
16-30	100±4.7	97±6.0	99±6.1	84±7.2	90±6.9	$F_{(4,54)}$=1.4;p=0.24
31-45	100±2.3	98±3.4	95±3.5	**84±4.5**	87±6.5	$F_{(4,54)}$=3.1;p=0.03
46-60	100±3.7	96±5.0	94±5.5	86±4.0	89±6.5	$F_{(4,54)}$=1.5;p=0.21
MAX Response Rate						
Allo Dose (mg/kg)	*Veh*	*3.0*	*5.6*	*10.0*	*17.0*	*ANOVA*
Time (min)						
0-15	100±23	114±12	114±15	124±13	114±14	$F_{(4,54)}$=0.29;p=0.88
16-30	100±20	121±9.1	127±8.9	157±18	124±12	$F_{(4,54)}$=1.9;p=0.12
31-45	100±19	112±6.9	127±12	148±12	125±15	$F_{(4,54)}$=1.7;p=0.16
46-60	100±19	103±6.2	116±4.8	145±12	116±8.0	$F_{(4,54)}$=2.5;p=0.06

All data are expressed as Mean ± SEM percent of vehicle injection. F-tests from one-way repeated measures ANOVA are indicated for each time interval. **Emboldened** values denote statistical significance from vehicle injection ($p<0.05$).

Psychopharmacology (Berl). Author manuscript; available in PMC 2015 December 28.