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Sex differences in sub-anesthetic ketamine's antidepressant effects and abuse liability

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Sub-anesthetic ketamine produces rapid antidepressant effects in patients with bipolar and unipolar major depression where conventional monoaminergic-based antidepressant drugs have been ineffective or ridden with side effects. A single ketamine infusion can produce antidepressant effects lasting up to two weeks, and multiple ketamine infusions prolong this effect. Pre-clinical studies are underway to uncover ketamine's mechanisms of action, but there are still many questions unanswered regarding the safety of its long-term use. Abuse liability is one area of concern, as recreational ketamine use is an ongoing issue in many parts of the world. Another understudied area is sex differences in responsivity to ketamine. Women are twice as likely as men to be diagnosed with depression, and they progress through stages of drug addiction more rapidly than their male counterparts. Despite this, preclinical studies in ketamine's antidepressant and addictive-like behaviors in females are limited. These intersecting factors in recent clinical and pre-clinical studies are reviewed to characterize ketamine's therapeutic potential, its limitations, and its potential mechanisms of action.

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Introduction

Major depressive disorder is a multi-symptom condition that contributes significantly to the global burden of disease, and women have a twofold higher risk of depression than men [1]. Treatment efforts have been hindered by monoaminergic-based antidepressants that have undesirable side effects and poor efficacy [2]. Therefore, the discovery that ketamine produces rapid antidepressant effects has generated much optimism in the field of psychiatry and neuroscience. While classical antidepressants take several weeks of daily administration to produce an effect, sub-anesthetic ketamine infusions alleviates depressive symptoms hours after the first infusion, lasting approximately 7–14 days in patients with treatment-resistant depression [3]. Furthermore, ketamine rapidly alleviates suicidal ideation, conferring a unique use of ketamine in the emergency room [4]. These findings, however, must be tempered by a number of notable limitations in our knowledge, especially that which pertains to the safety and efficacy of chronic long-term ketamine treatment, which has not been sufficiently addressed in clinical trials [5••]. Indeed, consequences of long-term exposure to ketamine have only been assessed in recreational contexts, as ketamine is used as a popular club drug because of its euphoric effects and vivid dissociative hallucinations. Importantly, recreational ketamine use differs greatly from antidepressant ketamine in terms of dose (higher), route of administration (typically intranasal), and setting (a rave or club). Nevertheless, few studies have assessed sex differences in ketamine responsivity. The inclusion of sex as a factor improves the translatability of preclinical research, and it may uncover important sex differences with implications for the abuse liability of antidepressant ketamine. Therefore, the purpose of this review is to bridge clinical and preclinical research to summarize what is known and to identify knowledge gaps regarding sex differences in ketamine response within the context of depression and recreational ketamine use.

Sex differences in stress and ketamine's antidepressant effects

While females are included in clinical research, few studies investigating ketamine's antidepressant effects analyze sex as a variable. A recent meta-regression analysis of six studies (n = 103) found that sex, age, and drug use history did not contribute to ketamine efficacy [6]. A larger meta-analysis analyzed 21 studies (n = 437) at four different time points, and they found no effect of sex at the 4 hours and 24 hours time points; only at the 7 day time point was having a greater proportion of male subjects predictive of overall higher efficacy [7]. These findings are limited to a relatively short time frame and only tested one acute ketamine dose (0.5 mg/kg/40 min intravenous infusion). To date, no clinical studies have looked at dose-dependent differences in ketamine efficacy in men and women, nor has repeated ketamine infusions been analyzed by sex.

Preclinical studies indicate a heightened sensitivity to ketamine in females. Stress-naïve female rodents consistently respond to a lower dose of ketamine than males on behavioral assays related to depression: specifically using measures of antidepressant efficacy (forced-swim test) and anxiety-induced neophagia (novelty-suppressed feeding) [8•,9–11], summarized in Table 1. The assessment of ketamine's effects on anhedonia, a signature feature of depression that is measured in rodents using the sucrose preference test, has produced conflicted findings. This is in part due to sex differences in baseline sucrose intake and ceiling effects [8•]. Discussed in a review by Kokras and Dalla [12], methodological factors such as duration of access to the bottles differentially affect the sucrose intake of males and females, thereby complicating interpretations of sex differences in depression-like behaviors. Non-consummatory measures of anhedonia such as intracranial self-stimulation of the lateral hypothalamus have shown that ketamine does not have an anti-anhedonic effect on socially defeated male mice while it rescues other depression-like behaviors induced by the social defeat stress [13]. Other models that utilize chronic stressors such as social isolation have found that female rats appear to be more resilient to social isolation stress, as they do not display a decrease in sucrose preference after 8 weeks of social isolation, which is sufficient to induce anhedonia in males [14••]. When chronic ketamine is tested (10 mg/kg daily, for 21 days), males displayed an antidepressant-like phenotype but females showed pro-depressive and anxiogenic behavioral traits [15•]. This study highlights the importance of including females in these studies, and furthermore it illustrates that sex differences can emerge following exposure to various drug dosages and treatment regimens, or exposure to different types of stressful stimuli. Another recent example of this comes from Hodes *et al.* [16], where subchronic social defeat stress (which produces a depression-like behavioral profile in female mice but not males, lending this model good face validity) results in a distinct pattern of gene expression in the nucleus accumbens of females compared to males, specifically differential expression of genes that control the DNA methylation machinery. The epigenetic mechanisms underlying sex-specific stress and ketamine responsiveness may be a compelling target of future research.

Ketamine's potential mechanisms of action

Antidepressant ketamine's putative mechanism of action is due to a rapid alteration of corticolimbic signaling and structural remodeling; however, the omission of females in these studies raises questions as to the translatability of these findings, as there has since been evidence that ketamine's mechanism may diverge in a sex-specific manner. In males, ketamine results in disinhibition of excitatory neurons in the medial prefrontal cortex (mPFC) via blockade of the n-methyl-D-aspartate (NMDA) receptors on inhibitory interneurons; this increased excitation leads to activation of downstream signaling cascades like mammalian target of rapamycin (mTOR) via Akt phosphorlylation, and the rapid synthesis of proteins that ultimately promotes synaptogenesis and increased spine density, thereby reversing effects of chronic stress [17]. In line with this, males that underwent chronic social isolation showed decreased sucrose preference, mPFC spine density, and expression of synaptic proteins postsynaptic density protein 95 (PSD-95), synapsin, and GluA1 [14••]. All effects were reversed with acute ketamine 3 hours later. Females, on the other hand, did not show a stressed-induced decrease in sucrose preference; they did show stressed-induced decreases in mPFC spine density and synaptic proteins, but ketamine did not rescue these alterations [14••]. Sex differences in glutamate neurotransmission have also been reported, where ketamine-treated males show increased hippocampal glutamate and females had no differences; while in the mPFC females had increased aspartate levels and males had no differences [9]. Additionally, increased hippocampal serotonin turnover was observed in females but not males [9].

Circulating gonadal hormones may underlie the female ketamine response in rodents. Indeed, female rats that undergo gonadectomy, thereby depleting levels of circulating estradiol and progesterone, displayed increased anxiety-like and depression-like behaviors which were alleviated by a dose of ketamine shown to work in both males and females (10 mg/kg) [18]. At a lower dose that is

Table 1

Summary of recent findings investigating behavioral sex differences in ketamine response

Measure	Mode of delivery	Behavioral outcome	Citations
Forced-swim test	Acute, IP	♀ respond to lower dose than ♂; estrus cycle-dependent	[8•,9,13,14••]
Forced-swim test	Repeated, IP	♀ prodepressive; ♂ antidepressant	[15•]
Novelty-suppressed feeding	Acute, IP	♀ respond to lower dose than ♂	[8]
Sucrose preference test	Acute + repeated, IP	Mixed/conflicting results; hormone-dependent	[8•,9,14••,19]
Locomotor sensitization	Repeated, IP	♀ respond to lower dose than ♂	[26,27]
Conditioned place preference/avoidance	Repeated, IP	Mixed/conflicting results, dose-dependent	[26–28]
Self-administration	Repeated, IV	♀ in proestrus similar to ♂, but diestrus lower than either	[30•]

IP, intraperitoneal; IV, intravenous.

only effective in females (2.5 mg/kg), replacing estradiol and progesterone levels in gonadectomized females was necessary to produce ketamine's antidepressant-like effects [8•]. Another study from our lab using gonadectomized rats demonstrated that activational effects of estradiol and progesterone (but not testosterone) are necessary in females, but not males, for 2.5 mg/kg ketamine's prohedonic effects on the sucrose preference test [19]. Additionally, pharmacological agonism of the two nuclear estrogen receptors (but not a progesterone receptor agonist) promoted ketamine behavioral response on the forced-swim test in intact diestrus 1 female mice [11]. Together these findings suggest that estrogen may have both protective effects on mood and a facilitative effect on ketamine responsiveness, warranting consideration for clinical studies of estrogen as an adjuvant with ketamine.

There are also intriguing sex differences in the metabolism of ketamine, where female rodents have higher brain levels of ketamine metabolites norketamine and hydroxynorketamine (HNK) than males (Saland *et al.*, unpublished; [10]), suggesting that differential pharmacokinetics may underlie the behavioral and molecular effects observed. Additionally, administration of the metabolite (2S,6S; 2R,6R)-HNK produces NMDA receptor-independent antidepressant-like effects without ketamine's adverse side effects [10]; however, others have recently challenged this view [20,21]. Furthermore, the antidepressant-like effects of this metabolite were only tested in males, thereby limiting the implications of these findings [10,21]. In humans, higher plasma levels of (2S,6S; 2R,6R)-HNK were observed in females than males; however, plasma levels of this specific metabolite were not correlated with treatment nonresponse as it was for other ketamine metabolites such as (2S,5S;2R,5R)-HNK in bipolar patients [22,23]. The pharmacologic effects of ketamine's active metabolites may underlie the prolonged antidepressant effects that extend beyond ketamine's half-life, and sex-specific differences in these metabolites may contribute to the differences in responsiveness observed in pre-clinical findings. More research is needed to fully characterize the metabolic pathways of ketamine and the different pharmacologic effects of each metabolite.

Ketamine abuse/addiction and implications for mood disorders

Ketamine is a popular club drug worldwide, and a number of detrimental health consequences are associated with its long-term use, including the development of addiction, increased tolerance over time, uncontrollable cravings, and symptoms of withdrawal upon cessation in many cases [24]. As individuals who exclusively use ketamine are rare, most observational ketamine studies involve poly-drug users. Studies investigating recreational ketamine use have uncovered some notable trends with regard to sex differences and comorbidity with mood disorders. Female ketamine users self-report greater levels of cognitive impairment and greater withdrawal effects compared to males [25], suggesting a potential sex-specific sensitivity to ketamine's effects. Additionally, women who use ketamine along with other amphetamine-type drugs are more likely to suffer from mood disorders compared to women who only use amphetamine-type drugs, and more than males [26]. Ignoring sex, club drug users (including ketamine) are more prone to bouts of depression than 'hard' drug users (the authors defined as cocaine or heroin) [27]. This association between mood disorders and ketamine use could be explained by individuals self-medicating in an attempt to alleviate their depressive symptoms, or that long-term ketamine use exacerbates the symptoms. A recent study found decreased resting state thalamocortical functional connectivity in chronic ketamine users from a sample that was 80% male [28]. This decreased functional connectivity is thought to underlie the transition from drug use to uncontrollable addiction. Additionally, female ketamine users have higher depression scores than males and differential resting state functional connectivity of subregions of the prefrontal cortex, an area greatly implicated in the pathophysiology of depression. Specifically, subgenual anterior cingulate cortex (sgACC) connectivity to the superior temporal gyrus in males and dorsomedial prefrontal cortex in females was correlated with a higher depression score [29•]. More research is needed to understand the structural and functional brain changes in male and female ketamine users, especially as it pertains to risk of depression.

Preclinical studies used to characterize ketamine's addiction-like properties involve behavioral assays such as locomotor sensitization, conditioned place preference (CPP), and intravenous self-administration, and sex difference studies have recently started to emerge. In line with females' increased sensitivity to antidepressant effects of ketamine discussed above, female rats also show increased locomotor sensitization to intermittent (weekly and every other day, respectively) repeated ketamine at depression-relevant doses (2.5–10.0 mg/kg intraperitoneally, i.p.) [30•,31]. Locomotor sensitization after repeated exposure to drugs of abuse is indicative of plasticity in the reward circuitry that may underlie the transition to addiction. Interestingly in these studies, the same rats that displayed sensitization did not form a CPP to ketamine at any dose tested [30•,31]; in fact, females displayed a conditioned place *aversion* to 5.0 mg/kg. Together, these findings suggest that divergent mechanisms may underlie the locomotor-activating effects and the associative rewarding effects of ketamine. However another group testing higher ketamine doses (6–14 mg/kg, daily) found females displayed a greater CPP than males [32]. This study also found distinct urine metabolic profiles in males and females, warranting further research into sex-specific pharmacokinetics of ketamine.

As discussed above, ovarian hormones mediate responsivity to ketamine's pro-hedonic effects [19]. There is also evidence that the reinforcing effects of other drugs of abuse, like cocaine, are augmented by estrogen via its interactions with the dopaminergic reward circuitry [33]. Freely cycling female rats that have access to ketamine self-administration only on days when they are in proestrus (when estradiol and progesterone levels peak) maintain their ketamine intake at levels comparable to males, but females with ketamine access only during diestrus 1 (when estradiol and progesterone are low) fail to maintain their intake [34•]. This suggests that the peak of gonadal hormones that coincides with proestrus supports the reinforcing effects of ketamine. Additionally, adolescent female rats display a stronger locomotor response to ketamine than males, and this effect was not seen in preadolescent rats whose circulating gonadal hormones have not yet peaked [35]. It is important, however, to note that several factors likely contribute to this behavioral effect including developmental changes in brain structures as well as potential pharmacokinetic differences related to different developmental stages. While these findings add to the growing body of evidence suggesting a contribution of ovarian hormones in responsivity to ketamine, a more systematic approach (for example, [19]) must be taken to fully elucidate the activational and organizational effects of gonadal hormones on the reinforcing properties of ketamine.

Rodent models have been used to study the effects of stress (the single greatest predictor of depression) on addiction-like behavior for a variety of drugs [36], and ketamine is beginning to be studied in this context. A recent study utilized the bulbectomy model in male rats, which is a model of depression that potentiates cocaine addiction-like behavior. Interestingly, while bulbectomy increased ketamine self-administration, it did not potentiate relapse [37], suggesting a different mechanism at play. This bulbectomy paradigm has yet to be tested in females, but female rats are more sensitive to stress-induced reinstatement of alcohol [38], and females are more responsive to corticotropin releasing factor (CRF) signaling in the context of cocaine withdrawal [39]. Sex differences in CRF signaling as it relates to ketamine's effects have yet to be studied, but it may be an attractive mechanism by which to explain the intersection of stress and addiction.

Different molecular adaptations between acute ketamine and chronic self-administration of ketamine have shown that a single ketamine infusion increases hippocampal brain-derived neurotropic factor (BDNF), while a history of chronic ketamine self-administration decreased hippocampal BDNF [40]. This group also found Akt was oppositely regulated by single vs repeated ketamine. Akt signaling and subsequent phosphorylation of mTOR may be the critical mechanism by which ketamine exerts its effects in the prefrontal cortex and hippocampus, but this may be a sex-specific and region-specific effect, as ketamine's protracted effects on synaptic plasticity in the nucleus accumbens of males, a critical center of reward processing, occurs independently of mTOR activation [41]. Additionally, differential nucleus accumbens phosphorylation of the glutamate receptor GluA1 has been observed with a single injection (increased [41]) vs chronic self-administration (decreased [42]). As the aforementioned studies only include males, it will be critical to characterize these effects in females as well.

Conclusions and future directions

The discovery of ketamine's rapid antidepressant effects has invigorated the field of psychiatry and given hope to millions of patients and caretakers. However, little is known about the long-term effects of repeated exposure to ketamine, and the addiction field has demonstrated that the neurobiological effects of acute vs chronic ketamine result in very different molecular profiles. A question clinicians may have is: where is the threshold at which the benefits of therapeutic, antidepressant ketamine become overshadowed by its risk of addiction? As women are twice as likely to develop depression and progress through the stages of addiction faster than men, are they at an increased risk? Preclinical literature reviewed therein suggests that female rats are more sensitive to ketamine's effects, but more research is needed in preclinical and clinical studies to fully understand sub-anesthetic ketamine.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, Charlson FJ, Norman RE, Flaxman AD, Johns N, Burstein R, Murray CJ, Vos T: **Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010**. *Lancet* 2013, **382**:1575-1586 http://dx.doi.org/10.1016/s0140-6736(13)61611-6 PMID: 23993280.

2. Chekroud AM, Gueorguieva R, Krumholz HM, Trivedi MH, Krystal JH, McCarthy G: **Reevaluating the efficacy and predictability of antidepressant treatments: a symptom clustering approach**. *JAMA Psychiatry* 2017, **74**:370-378 http://dx.doi.org/10.1001/jamapsychiatry.2017.0025 PMID: 28241180.

3. Fond G, Loundou A, Rabu C, Macgregor A, Lancon C, Brittner M, Micoulaud-Franchi JA, Richieri R, Courtet P, Abbar M, Roger M, Leboyer M, Boyer L: **Ketamine administration in depressive disorders: a systematic review and meta-analysis**. *Psychopharmacology (Berl)* 2014, **231**:3663-3676 http://dx.doi.org/10.1007/s00213-014-3664-5 PMID: 25038867.

4. Wilkinson ST, Ballard ED, Bloch MH, Mathew SJ, Murrough JW, Feder A, Sos P, Wang G, Zarate CA Jr, Sanacora G: **The effect of a single dose of intravenous ketamine on suicidal ideation: a systematic review and individual participant data meta-analysis**. *Am J Psychiatry* 2017 http://dx.doi.org/10.1176/appi.ajp.2017.17040472. appiajp201717040472. PMID: 28969441.

5. •• Sanacora G, Frye MA, McDonald W *et al.*: **A consensus statement on the use of ketamine in the treatment of mood disorders**. *JAMA Psychiatry* 2017, **74**:399-405 http://dx.doi.org/10.1001/jamapsychiatry.2017.0080.
This important consensus statement from the American Psychiatric Association Council of Research Task Force on Novel Biomarkers and Treatments reviews the clinical research demonstrating ketamine's antidepressant effects, suggests best practices for physicians based on what is currently known, and emphasizes the critical limitations in the field.

6. Romeo B, Choucha W, Fossati P, Rotge JY: **Meta-analysis of short- and mid-term efficacy of ketamine in unipolar and bipolar depression**. *Psychiatry Res* 2015, **230**:682-688 http://dx.doi.org/10.1016/j.psychres.2015.10.032 PMID: 26548981.

7. Coyle CM, Laws KR: **The use of ketamine as an antidepressant: a systematic review and meta-analysis**. *Hum Psychopharmacol* 2015, **30**:152-163 http://dx.doi.org/10.1002/hup.2475 PMID: 25847818.

8. • Carrier N, Kabbaj M: **Sex differences in the antidepressant-like effects of ketamine**. *Neuropharmacology* 2013, **70**:27-34 http://dx.doi.org/10.1016/j.neuropharm.2012.12.009 PMID: 23337256.
This was the first work to demonstrate female rats respond to lower ketamine doses than males on tests of antidepressant-like behavior; while other studies have shown that increased mTOR phosphorylation in the mPFC and decreased eEF2 phosphorylation in the hippocampus underlie ketamine's antidepressant effects, neither were reported in females at the male sub-threshold dose of 2.5 mg/kg, suggesting an independent mechanism.

9. Franceschelli A, Sens J, Herchick S, Thelen C, Pitychoutis PM: **Sex differences in the rapid and the sustained antidepressant-like effects of ketamine in stress-naive and "depressed" mice exposed to chronic mild stress**. *Neuroscience* 2015 http://dx.doi.org/10.1016/j.neuroscience.2015.01.008. PMID: 25595985.

10. Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI, Alkondon M, Yuan P, Pribut HJ, Singh NS, Dossou KS, Fang Y, Huang XP, Mayo CL, Wainer IW, Albuquerque EX, Thompson SM, Thomas CJ, Zarate CA Jr, Gould TD: **NMDAR inhibition-independent antidepressant actions of ketamine metabolites**. *Nature* 2016 http://dx.doi.org/10.1038/nature17998. PMID: 27144355.

11. Dossat A, Wright K, Strong C, Kabbaj M: **Behavioral and biochemical sensitivity to low doses of ketamine: influence of estrous cycle in C57BL/6 mice**. *Neuropharmacology* 2018, **130**:30-41 http://dx.doi.org/10.1016/j.neuropharm.2017.11.022 PMCID: PMC5749639.

12. Kokras N, Dalla C: **Sex differences in animal models of psychiatric disorders**. *Br J Pharmacol* 2014, **171**:4595-4619 http://dx.doi.org/10.1111/bph.12710 PMID: 24697577; PMCID: PMC4209934.

13. Donahue RJ, Muschamp JW, Russo SJ, Nestler EJ, Carlezon WA Jr: **Effects of striatal DeltaFosB overexpression and ketamine on social defeat stress-induced anhedonia in mice**. *Biol Psychiatry* 2014, **76**:550-558 http://dx.doi.org/10.1016/j.biopsych.2013.12.014 PMID: 24495460; PMCID: 4087093.

14. •• Sarkar A, Kabbaj M: **Sex differences in effects of ketamine on behavior, spine density, and synaptic proteins in socially isolated rats**. *Biol Psychiatry* 2016 http://dx.doi.org/10.1016/j.biopsych.2015.12.025. PMID: 26957131.
In this study, socially isolated male rats treated with ketamine display a reversal of stress-induced induction of depression-like behavior, as well as reversal of stress-induced reductions in mPFC spine density and related proteins (Synapsin 1, PSD-95, and GluR1). This is in dramatic contrast to the females, where an expected behavioral effect of ketamine was demonstrated, but no ketamine reversal of decreased mPFC spine density and synaptic proteins. These findings suggest a divergent mechanism of action for ketamine in females.

15. • Thelen C, Sens J, Mauch J, Pandit R, Pitychoutis PM: **Repeated ketamine treatment induces sex-specific behavioral and neurochemical effects in mice**. *Behav Brain Res* 2016, **312**:305-312 http://dx.doi.org/10.1016/j.bbr.2016.06.041 PMID: 27343934.
Recent study looking at repeated ketamine exposure effects on mood-related behavior and molecular changes in mice. While 21 days of daily 10 mg/kg ketamine produced a sustained antidepressant effect in males, it was *pro*-depressive and anxiogenic in females. Additionally they found increased hippocampal synapsin 1 and SNARE proteins in males, but not in females.

16. Hodes GE, Pfau ML, Purushothaman I, Ahn HF, Golden SA, Christoffel DJ, Magida J, Brancato A, Takahashi A, Flanigan ME, Menard C, Aleyasin H, Koo JW, Lorsch ZS, Feng J, Heshmati M, Wang M, Turecki G, Neve R, Zhang B, Shen L, Nestler EJ, Russo SJ: **Sex differences in nucleus accumbens transcriptome profiles associated with susceptibility versus resilience to subchronic variable stress**. *J Neurosci* 2015, **35**:16362-16376 http://dx.doi.org/10.1523/jneurosci.1392-15.2015 PMID: 26674863; PMCID: Pmc4679819.

17. Abdallah CG, Adams TG, Kelmendi B, Esterlis I, Sanacora G, Krystal JH: **Ketamine's mechanism of action: a path to rapid-acting antidepressants**. *Depress Anxiety* 2016, **33**:689-697 http://dx.doi.org/10.1002/da.22501 PMID: 27062302; PMCID: PMC4961540.

18. Moreira SF, Nunes EA, Kuo J, de Macedo IC, Muchale A, de Oliveira C, Scarabelot VL, Marques Filho PR, Medeiros LF, Caumo W, Torres IL: **Hypoestrogenism alters mood: ketamine reverses depressive-like behavior induced by ovariectomy in rats**. *Pharmacol Rep* 2016, **68**:109-115 http://dx.doi.org/10.1016/j.pharep.2015.06.009 PMID: 26721361.

19. Saland SK, Schoepfer KJ, Kabbaj M: **Hedonic sensitivity to low-dose ketamine is modulated by gonadal hormones in a sex-dependent manner**. *Sci Rep* 2016, **6**:21322 http://dx.doi.org/10.1038/srep21322 PMID: 26888470; PMCID: Pmc4766854.

20. Suzuki K, Nosyreva E, Hunt KW, Kavalali ET, Monteggia LM: **Effects of a ketamine metabolite on synaptic NMDAR function**. *Nature* 2017, **546**:E1-E3 http://dx.doi.org/10.1038/nature22084 PMID: 28640258.

21. Yang C, Qu Y, Abe M, Nozawa D, Chaki S, Hashimoto K: **(R)-ketamine shows greater potency and longer lasting antidepressant effects than its metabolite (2R,6R)-hydroxynorketamine**. *Biol Psychiatry* 2017, **82**:e43-e44 http://dx.doi.org/10.1016/j.biopsych.2016.12.020 PMID: 28104224.

22. Zarate CA Jr, Brutsche N, Laje G, Luckenbaugh DA, Venkata SL, Ramamoorthy A, Moaddel R, Wainer IW: **Relationship of ketamine's plasma metabolites with response, diagnosis, and side effects in major depression**. *Biol Psychiatry* 2012, **72**:331-338 http://dx.doi.org/10.1016/j.biopsych.2012.03.004 PMID: 22516044; PMCID: PMC3442255.

23. Abdallah CG: **What's the buzz about hydroxynorketamine? Is it the history, the story, the debate, or the promise?**. *Biol Psychiatry* 2017, **81**:e61-e63 http://dx.doi.org/10.1016/j.biopsych.2017.01.002 PMID: 28317551; PMCID: PMC5484143.

24. Morgan CJ, Curran HV: **Ketamine use: a review**. *Addiction* 2012, **107**:27-38 http://dx.doi.org/10.1111/j.1360-0443.2011.03576.x PMID: 21777321.

25. Chen WY, Huang MC, Lin SK: **Gender differences in subjective discontinuation symptoms associated with ketamine use**. *Subst Abuse Treat Prev Policy* 2014, **9**:39 http://dx.doi.org/10.1186/1747-597x-9-39 PMID: 25245125; PMCID: Pmc4183767.

26. Zhang Y, Lu C, Zhang J, Hu L, Song H, Li J, Kang L: **Gender differences in abusers of amphetamine-type stimulants and ketamine in southwestern China**. *Addict Behav* 2013, **38**:1424-1430 http://dx.doi.org/10.1016/j.addbeh.2012.06.024 PMID: 23006246.

27. Chen WJ, Wu SC, Tsay WI, Chen YT, Hsiao PC, Yu YH, Ting TT, Chen CY, Tu YK, Huang JH, Yang HJ, Li CY, Strong C, Yen CF, Hsu J: **Differences in prevalence, socio-behavioral correlates, and psychosocial distress between club drug and hard drug use in Taiwan: results from the 2014 National Survey of Substance Use**. *Int J Drug Policy* 2017, **48**:99-107 http://dx.doi.org/10.1016/j.drugpo.2017.07.003 PMID: 28810160.

28. Liao Y, Tang J, Liu J, Xie A, Yang M, Johnson M, Wang X, Deng Q, Chen H, Xiang X, Liu T, Chen X, Song M, Hao W: **Decreased**

thalamocortical connectivity in chronic ketamine users. *PLOS ONE* 2016, **11**:e0167381 http://dx.doi.org/10.1371/journal.pone.0167381 PMID: 27977717; PMCID: PMC5157971.

29. Li CR, Zhang S, Hung CC, Chen CM, Duann JR, Lin CP, Lee TS:
• **Depression in chronic ketamine users: sex differences and neural bases**. *Psychiatry Res* 2017, **269**:1-8 http://dx.doi.org/10.1016/j.pscychresns.2017.09.001 PMID: 28892733.
Female ketamine users show greater functional connectivity of the sgACC to the dorsomedial prefrontal cortex, while males have greater connectivity of the sgACC to the superior temporal gyrus, both associated with higher depression scores. These differences in functional connectivity may underlie the sex differences seen in depressed individuals not using ketamine.

30. Strong CE, Schoepfer KJ, Dossat AM, Saland SK, Wright KN, Kabbaj M: **Locomotor sensitization to intermittent ketamine administration is associated with nucleus accumbens plasticity in male and female rats**. *Neuropharmacology* 2017, **121**:195-203 http://dx.doi.org/10.1016/j.neuropharm.2017.05.003 PMID: 28479397; PMCID: PMC5520991.
Females have increased sensitivity to ketamine on measures of addiction-like behavior. Additionally, this was the first study to investigate changes in dendritic spine density in the NAc core and shell: while both males and females had increased spine density in the shell, only females had an increase in the core.

31. Schoepfer KJ, Strong CE, Saland SK, Wright KN, Kabbaj M: **Sex- and dose-dependent abuse liability of repeated subanesthetic ketamine in rats**. *Physiol Behav* 2017 http://dx.doi.org/10.1016/j.physbeh.2017.10.021. PMID: 29055748.

32. Guo R, Tang Q, Ye Y, Lu X, Chen F, Dai X, Yan Y, Liao L: **Effects of gender on ketamine-induced conditioned placed preference and urine metabonomics**. *Regul Toxicol Pharmacol* 2016, **77**:263-274 http://dx.doi.org/10.1016/j.yrtph.2016.03.007 PMID: 26995028.

33. Bobzean SA, DeNobrega AK, Perrotti LI: **Sex differences in the neurobiology of drug addiction**. *Exp Neurol* 2014, **259**:64-74 http://dx.doi.org/10.1016/j.expneurol.2014.01.022 PMID: 24508560.

34. Wright KN, Strong CE, Addonizio MN, Brownstein NC, Kabbaj M:
• **Reinforcing properties of an intermittent, low dose of ketamine in rats: effects of sex and cycle**. *Psychopharmacology (Berl)* 2017, **234**:393-401 http://dx.doi.org/10.1007/s00213-016-4470-z PMID: 27837330; PMCID: PMC5384643.
In this study, the reinforcing effects of ketamine were found to be supported by co-exposure of endogenous estradiol and progesterone in proestrus females, as diestrus-trained females fail to maintain ketamine self-administration. Additionally, this study found that ketamine-seeking behaviors require the presence of drug-paired cues, as ketamine alone does not trigger reinstatement.

35. McDougall SA, Moran AE, Baum TJ, Apodaca MG, Real V: **Effects of ketamine on the unconditioned and conditioned locomotor activity of preadolescent and adolescent rats: impact of age, sex, and drug dose**. *Psychopharmacology (Berl)* 2017 http://dx.doi.org/10.1007/s00213-017-4660-3. PMID: 28589265.

36. Ng E, Browne CJ, Samsom JN, Wong AH: **Depression and substance use comorbidity: what we have learned from animal studies**. *Am J Drug Alcohol Abuse* 2016:1-19 http://dx.doi.org/10.1080/00952990.2016.1183020. PMID: 27315335.

37. Babinska Z, Ruda-Kucerova J: **Differential characteristics of ketamine self-administration in the olfactory bulbectomy model of depression in male rats**. *Exp Clin Psychopharmacol* 2017 http://dx.doi.org/10.1037/pha0000106. PMID: 28301174.

38. Bertholomey ML, Nagarajan V, Torregrossa MM: **Sex differences in reinstatement of alcohol seeking in response to cues and yohimbine in rats with and without a history of adolescent corticosterone exposure**. *Psychopharmacology (Berl)* 2016, **233**:2277-2287 http://dx.doi.org/10.1007/s00213-016-4278-x PMID: 27048157.

39. Cason AM, Kohtz A, Aston-Jones G: **Role of corticotropin releasing factor 1 signaling in cocaine seeking during early extinction in female and male rats**. *PLOS ONE* 2016, **11**: e0158577 http://dx.doi.org/10.1371/journal.pone.0158577 PMID: 27362504; PMCID: PMC4928795.

40. Caffino L, Chio MD, Giannotti G, Venniro M, Mutti A, Padovani L, Cheung D, Fumagalli GF, Yew DT, Fumagalli F, Chiamulera C: **The modulation of BDNF expression and signalling dissects the antidepressant from the reinforcing properties of ketamine: effects of single infusion vs. chronic self-administration in rats**. *Pharmacol Res* 2015 http://dx.doi.org/10.1016/j.phrs.2015.12.014. PMID: 26706783.

41. Yao N, Skiteva O, Zhang X, Svenningsson P, Chergui K: **Ketamine and its metabolite (2R,6R)-hydroxynorketamine induce lasting alterations in glutamatergic synaptic plasticity in the mesolimbic circuit**. *Mol Psychiatry* 2017 http://dx.doi.org/10.1038/mp.2017.239. PMID: 29158578.

42. Caffino L, Piva A, Mottarlini F, Di Chio M, Giannotti G, Chiamulera C, Fumagalli F: **Ketamine self-administration elevates alphaCaMKII autophosphorylation in mood and reward-related brain regions in rats**. *Mol Neurobiol* 2017 http://dx.doi.org/10.1007/s12035-017-0772-3. PMID: 28948570.