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REVIEW

Ketamine – More mechanisms of action than just NMDA blockade

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SUMMARY

Ketamine has been in clinical use for over half a century, yet its precise mechanisms of action remain mysterious for the large part. Its hypnotic effects appear to be largely mediated by blockade of NMDA and HCN1 receptors, but cholinergic, aminergic, and opioid systems appear to play both a positive and negative modulatory role in both sedation and analgesia. Ketamine's effects in chronic pain, and as an antidepressant, far outlast the actual drug levels, and are probably mediated by a secondary increase in structural synaptic connectivity that is mediated by a neuronal response to the ketamine-induced hyperglutamatergic state.

1. Introduction

Ketamine is a well-established anaesthetic drug that has been in use for around 50 years.[1] It produces a spectrum of anaesthetic effects that results in a type of anaesthesia that has an obviously different feel qualitatively as compared to more traditional volatile-based anaesthesia. This state of so-called "dissociative anaesthesia" has been well described as including: (a) hypnosis – which includes psychotomimetic effects at low concentrations, followed by increasing sedation and unconsciousness at higher doses; (b) intense analgesia (or more accurately anti-nociception); (c) increased sympathetic activity; and (d) maintenance of airway tone and respiration. It has been known since the mid 1980s that ketamine caused use-dependent blockade of the N-methyl-D-aspartate (NMDA) receptor[2]; and that this blockade of excitatory synaptic activity probably caused the loss of responsiveness that is associated with clinical ketamine anaesthesia. However, in the intervening years, subsequent work has demonstrated that ketamine exhibits a wide range of different molecular effects, and its clinical usefulness has expanded to include a role in the management of a wide range of conditions including acute and chronic pain,[3] and, most recently, as a rapidly acting antidepressant.[4] Intriguingly many of these clinically beneficial effects seem to occur long after the actual drug has been almost completely excreted from the body. Clearly the relationship between the drug's binding and its clinical effects is more complex than first realised.

Linking observed molecular actions for any particular drug with its clinical effects is an abiding pharmacological problem. We would propose that the following set of axioms can be used as a guide to decide whether a particular mechanism is a necessary and sufficient cause of an observed behaviour.

1) The molecular or cellular effects occur at a similar concentration to those which are associated with behavioural changes – and a reasonable dose–response curve should be defined.
2) All the drugs which have been observed to cause a particular molecular or cellular effect should cause the associated behavioural change. There is a proviso; if some drug has a cellular effect in the laboratory, but none in vivo, there must be a reasonable explanation for this discrepancy – such as inability to penetrate the blood–brain barrier in sufficient concentrations, or the presence of drug metabolism or extrusion enzymes.
3) The co-administration of agonists or antagonists should modulate the dose–response curve of the drug. Again there is a proviso – which is quite common with anaesthetic drugs, which tend to be allosteric modulators of receptor or ion channel function. Unless there exists a pure competitive antagonist compound, it is likely that these effects will be modest.
4) Other non-drug interventions such as genetic (knockout, or knockin, full or conditional) or mimetic peptides which produce the same changes in neuronal mechanisms, as are seen with the drug, should produce the same behavioural effects as the drug.

Using these principles for guidance, we will briefly summarise the known molecular effects of ketamine, and then discuss the relevant importance of each on the known clinical actions of the

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drug. As will be seen in subsequent paragraphs, even after 50 years of research, there are still many question marks over exactly which of the observed molecular actions of ketamine are significant in causing the various observed components of anaesthesia in patients. The aim of this review is to provide a broad summary of the information relating to ketamine's actions and effects. It does not have the space to touch on the other effects of ketamine on inflammation, apoptosis, or in neuronal excitotoxicity. For further in-depth reviews, we would draw the readers' attention to a recent issue of CNS Neuroscience & Therapeutics that was devoted to examining the various roles of ketamine in modern medicine.[5]

2. Molecular actions of ketamine

2.1. Immediate effects

At concentrations within the clinical dose range, ketamine is now known to directly affect a wide range of cellular processes – including blockade of NMDA channels, neuronal hyperpolarisation-activated cationic currents (Ih, also known as hyperpolarisation-activated cyclic nucleotide channels (HCN1)), nicotinic acetyl-choline ion channels, delta and mu-opioid agonism and opioid potentiation,[6] the nitric-oxide (NO)–cyclic guanosine-mono-phosphate (cGMP) system, non-NMDA glutamate receptors (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)), and metabotropic glutamate receptors (mGluR), reduction in cholinergic neuromodulation,[7,8] increased release of aminergic neuromodulators (dopamine and noradrenaline),[9–11] neurosteroids,[12] and L-type Ca2+ channels.[13] Of course all these different systems do not act in isolation, but are themselves part of the integrated nervous system with a myriad of interactions occurring at all levels.[14]

2.2. How does ketamine disrupt NMDA channel function?

At the level of the chemical binding of the NMDA antagonistic drug with the NMDA receptor, the exact processes by which various groups of compounds exert an impairment of NMDA receptor function are quite complex and reviewed in detail in.[15–17] There are numerous known compounds that influence NMDA action. They can be broadly classified as: (a) competitive antagonists, (b) open channel blockers (ketamine is one of the least potents), (c) non-competitive antagonists, and (d) allosteric potentiators. All these compounds have differing relative potency on the different NMDA receptor subtypes (commonly termed GluN1, GluN2A, GluN2B, GluN2C, and GluN2D – but also called NR1, NR2A-D) – with resultant different spectra of action. These subtypes show markedly heterogeneous distributions in the brain, which may account for the variations in clinical effects caused by different NMDA blocking compounds. The GluN2A subtype is found throughout the brain, whereas GluN2B is primarily confined to the limbic system, thalamus, and spinal cord, GluN2C to the thalamus and cerebellum, and GluN2D in the brain stem, diencephalon, and spinal cord.

Another important reason for the variation in effect lies in the off-rate of the compound. This phenomenon has been termed "trapping block".[18,19] Compounds with a slow off-rate such as ketamine (86% trapping) and MK-801 (almost 100% trapping) are examples of high-trapping antagonists. When the glutamate has dissociated from its binding site on the NMDA receptor, the ketamine remains trapped in the, now closed, ion channel thus causing a prolonged tonic blockade which disrupts both physiological and pathological function. In contrast low-trapping (fast off-rate) antagonists are able to escape from the channel before it closes, thus allowing some preservation of physiological NMDA function, and hence fewer side effects. For example the compound memantine (50–70% trapped) has minimal sedative or psychotomimetic effects. It is a low affinity open-channel blocker with a fast off-rate. It therefore only blocks NMDA channels which are open for a pathologically prolonged time, and has minimal effect when the NMDA channel is only transiently open, as occurs in most physiological states. In many ways this mechanism is analogous to the effect of persistent sodium channel blocking antiepileptic drugs.[20] The net result is an NMDA blocker without appreciable anaesthetic effects.

2.3. Delayed effects of ketamine

Cell function consists of much more than ion channel function. Each of the immediate effects of ketamine disrupts a large number of downstream, and more longer lasting, cellular processes, such as altered gene expression and protein regulation. Perhaps this is not surprising, given the pivotal importance of NMDA control of calcium entry into the cell, and the many intracellular effects of calcium ions on protein and mitochondrial function.

Ketamine has been shown to result in suppression of immediate early gene expression at the site of mechanical injury (zif/268, c-fos, junB, fosB, c-jun, junD).[21] It also alters the regulation of NMDA receptor1 phosphorylation[22] and NMDA receptor1 mRNA expression[23] in rat and mouse models of hyperalgesia, and also limits astrocytic and microglial activation as seen in reduced glial fibrillary acidic protein (GFAP) expression[24,25]; effects that correlate with a reduction in neuropathic pain. While these chronic pain models reflect slow evolving patterns of nociception, similar actions may be relevant to acute pain. Ketamine has also been shown to enhance brain-derived neurotrophic factor (BDNF) and mammalian target of rapamycin (mTOR) protein levels in the rat hippocampus,[26,27] resulting in modification to the number and function of synaptic connections.

A simple pictorial representation of the effects of ketamine is shown in Fig. 1. We will now examine each clinical effect in more detail.

3. Psychotomimesis

At ketamine concentrations of around 50–100 ng/ml, most patients will report feelings of dissociation and even suffer florid psychotic symptoms of hallucinations and delirium. These symptoms are usually attributed directly to the NMDA blockade, as it is a prominent feature of most NMDA antagonist compounds. However there are a small set of NMDA-blocking drugs in which the psychotomimetic effects seem to be curiously minimal. Whilst some of this may be explicable by differences in the "trapping block" as described previously, it is likely that other mechanisms contribute significantly to the potential to induce hallucinations.

In recent years it has become clear that ketamine disturbs the feedback mechanisms that control synaptic homoeostasis – either by modifying neurotransmitter release or re-uptake, or background neuromodulator tone. One interesting possibility is the effect of the ketamine in reducing the ability of NADPH oxidase (NOX2) to control glutamate release.[28] A relative overabundance of glutamate activity has been associated with psychosis. However, an alternative, or perhaps complementary, explanation is that the ketamine has disturbed 'Regulator of G protein signalling 4' (Rgs4); which is a signal transduction protein that controls the function of monoamine, opiate, muscarinic, and other G protein-coupled receptors.[29] It is well known that ketamine's effects in increasing dopaminergic activity,[11] and possibly a net decrease in acetyl choline activity[7] will act together to make delirium more pronounced.

4. Hypnosis

Loss of responsiveness to the outside world occurs at about twenty-fold higher concentrations (around 2000 ng/ml) of

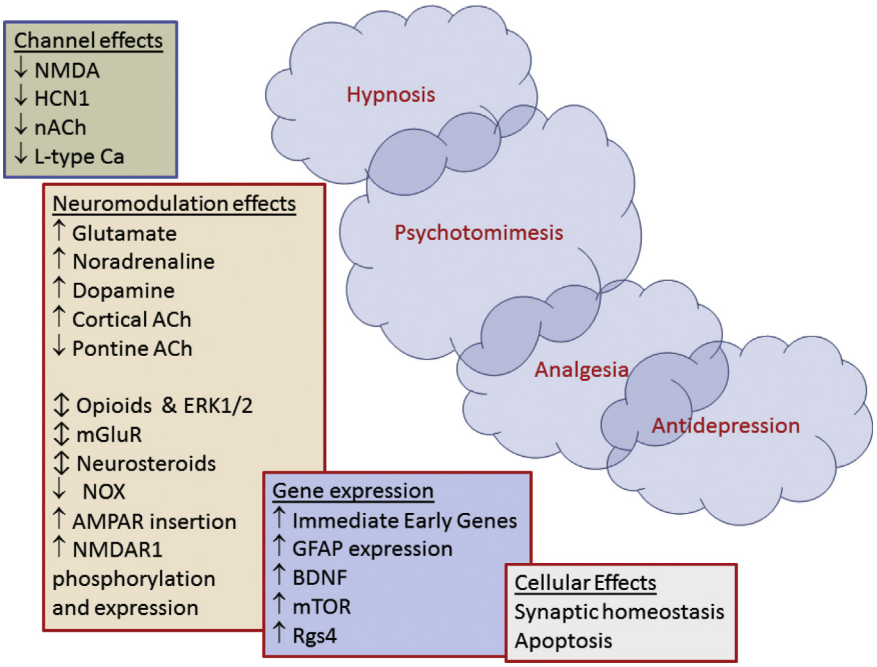

Fig. 1. Summary diagram of the neuropharmacological actions of ketamine, and the resultant clinical effects. The rapid effects and actions are represented at the top left, and the more delayed and prolonged effects and actions represented towards the bottom right. [NMDA = N-methyl-D aspartate, HCN1 = hyperpolarisation-activated cyclic nucleotide channels, ACh = acetyl choline, nACh = nicotinic acetyl-choline receptors, AMPA = α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid, mGluR = metabotropic glutamate receptors, ERK1/2 = extracellular signal-regulated kinases, NOX = NADPH oxidase, BDNF = brain-derived neurotrophic factor, mTOR = mammalian target of rapamycin, Rgs4 = regulator of G protein signalling 4, L-type Ca2 = L-type calcium channels, GFAP = glial fibrillary acidic protein].

ketamine than those required to induce the psychotomimetic effects. Since the elimination half-life of ketamine is around 3 h,[30] there is a prolonged period, after anaesthesia, in which the drug levels are lying in the concentration range that produces psychotomimesis.[31] We should also note that the duration of hypnosis strictly follows the time course of the blood (and effect-site) concentrations of the drug; which indicates that secondary slow effects do not play an important causative role in the hypnosis/anaesthesia state. Ketamine is unique amongst commonly used anaesthetic drugs in that it demonstrates a curious mixture of depressant (such as NMDA antagonism) and analeptic (increase in amines, glutamate excess, and increased AMPA receptor insertion) molecular actions. For this reason it is hard to achieve full anaesthesia in many species, and in veterinary anaesthesia, ketamine is commonly co-administered with an alpha-2 adrenergic agonist to achieve surgical anaesthesia. It has been suggested that the central nervous system depression that is seen with ketamine anaesthesia occurs because the NMDA receptors found on the dendrites of the inhibitory neuronal populations are less sensitive to the effects of ketamine than those located on the excitatory neurons. The net result is that ketamine preferentially inhibits excitatory-to-excitatory coupling above inhibitory-to-excitatory coupling.[32]

Although the anaesthesia is commonly attributed to the NMDA blockade, it is likely that other molecular actions play an important role in hypnosis. Evidence for this comes from a variety of sources. Firstly, hypnotic potency does not correlate well with potency for NMDA blockade. It is clear that many compounds which are much more potent and are more specific NMDA blockers – such as dizocilpine maleate (MK-801) and dextrorphan – are only very weakly hypnotic.[33] One possible explanation for this difference between ketamine and MK-801, is that ketamine has a relatively higher potency for GluN2C receptors which would – in theory – induce relatively more thalamic hyperpolarisation than a drug which has greater potency on the GluN2A and B receptors – such as MK-801. However the counter example is memantine, which has a comparable affinity for GluN2C receptors as ketamine, but is not clinically sedative. Whether this discrepancy can be accounted for by the marked difference in strength of trapping-block between ketamine and memantine, is not known.

Secondly, it might be expected that NMDA knockout animals might be completely resistant to ketamine. Petrenko and co-workers have studied knockout mice who were deficient in the GluEpsilon1subchain of the NMDA receptor.[34] They found that these animals were indeed resistant to hypnosis with ketamine. However these animals were also resistant to a non-NMDA blocking anaesthetic agent pentobarbitone – which suggests a non-specific analeptic effect in these animals. The authors concluded that the decreased ketamine sensitivity in the knockout animals was not caused by the genetic knockout of the NMDA receptor, but rather, was mediated via a compensatory secondary increase in monoaminergic tone[35] – which would reduce the tendency towards hypnosis.

Recent work has also implicated other families of receptors in the hypnotic effects of ketamine. In particular, a very clean model – which used conditional forebrain knock-out mice for the HCN1 channel – showed a 30% reduction in the hypnotic potency of ketamine.[36,37] The role of brain stem sleep centres in the hypnotic actions of ketamine is unclear and somewhat paradoxical.[38] The drug has been shown to promote a slow wave sleep pattern by decreasing cholinergic activity in the pons.[7] In contrast the drug directly or indirectly augments neocortical aminergic[9] and cholinergic[39] activity which should promote wakefulness.

5. Analgesia

Ketamine will reduce pain scores in concentrations close to those that produce psychotomimetic effects (~200 ng/ml). Like the hypnotic and analeptic effects discussed above, ketamine produces

a complex mixture of both anti- and pro-nociceptive actions. The place of ketamine analgesia in clinical practice is still subject to much debate.[40,41] There are many conflicting data, and the analgesic effects of ketamine have to be carefully interpreted in the light of specifically diagnosed pain syndromes.[42] It is of note that norketamine has been shown to actually have anti-analgesic effects,[43] and ketamine may actually facilitate endogenous pain pathways[44] in some circumstances. The widespread use of the drug is somewhat limited by the narrow therapeutic window between analgesia and excessive sedation or psychotomimetic effects. In the main, the analgesic effects are correlated with drug levels – based on direct receptor mediated analgesia mechanisms. However in the setting of chronic neuropathic pain syndromes, there is some evidence for prolonged post-drug analgesia that markedly outlasts the effective drug levels, which would be mediated by downstream mechanisms.[40,45,46]

It is generally considered that supraspinal blockade of the NR2B NMDA sub-unit has the most important anti-nociceptive influence[47]; but ketamine also has direct effects on the delta opioid receptor, and acts to augment opioid mu-receptor function.[48,49] Ketamine certainly modifies responsiveness to opioid receptors. However its analgesia is not reduced by naloxone; which would argue against the importance of primary opioid mechanisms of action. In support of the non-opioid mechanisms of analgesia hypothesis, studies using knockout mice for the G protein-coupled inwardly rectifying potassium channels (GIRK2s) have suggested that function of this channel was pivotal in the pathway by which clonidine and opioids exerted a significant part of their analgesia. In contrast the analgesic effect of ketamine seems to be largely independent of this pathway[50]; and rather, the effects were associated with increased dopamine activity in these mice. The interactions between ketamine and the opioid system are probably more relevant in chronic pain, where ketamine reduces opioid tolerance. In vitro Gupta and colleagues found that ketamine prevented and even reversed opioid mu-receptor desensitisation, acting downstream of the receptor on the amount of ERK1/2 phosphorylation.[51]

Ketamine also augments endogenous anti-nociceptive systems – presumably, in part, via its aminergic (serotonergic and noradrenergic) activation and inhibition of re-uptake.[52] Ketamine also directly inhibits nitric-oxide synthase which probably contributes in part to its analgesic effects,[53] although the relative importance of these mechanisms has not been well quantified to date.

5.1. Control of chronic pain

Ketamine has both acute and prolonged effects on chronic neuropathic pain syndromes. A single administration of a low analgesic dose (250 μg/kg) can rapidly (5–10 min) and transiently (2–3 h) reduce ongoing pain of neuropathic origin, as well as symptoms of allodynia and hyperalgesia.[54] The latter may be attributable to a reduction in NMDA-mediated "wind-up".[55] However, these effects are not consistent from individual to individual. Even within the same subject group, transient (<2 h), enduring (6–24 h) and no analgesic effect may be reported.[46] Ketamine applied around the time of surgery as a single infusion has even been reported to limit the development of chronic pain 30–180 days postoperatively.[56]

On the surface, some of these findings appear to contradict the clinical observation that ketamine lacks long-lasting analgesic properties. One explanation is that ketamine's pre-emptive reduction in neuropathic pain is a corollary of its antidepressant effect[57,58] — which endures well after the drug has been eliminated.[58] Chronic pain and depression are often closely linked,[59] although the direction of the causative relationship between the two is less clear. Alternatively, ketamine may set in chain cell signalling cascades that interrupt the gradual propagation of pathophysiological changes associated with chronic pain development.[60] As outlined in a previous section, ketamine regulates a number of gene expression pathways potentially linked to chronic pain aetiology, including NMDA receptor expression, astrocytic activation and synaptic structure and function. These effects would long outlast the detectable presence of the drug.

5.2. Antidepressant effects

There has been much recent interest in the use of ketamine as a 'rapidly acting' antidepressant. However in this context the time-of-onset is still around 2 h, and the duration of antidepressant effect lasts about a week. These effects clearly occur long after the drug has been eliminated from the body – which is indicative of a ketamine-induced signalling cascade.[61] The putative mechanisms have been reviewed in detail by Duman and co-workers[4]; in brief they suggest that the ketamine at low doses actually increases glutamate neurotransmission by both increased glutamate release and increased AMPA receptor expression and insertion into the synaptic plate. This causes secondary increased BDNF release, and hence activation of ERK signalling which then stimulates mammalian target of rapamycin (mTOR) – a kinase that controls protein translation – and thus via a complex signalling path, leads to increased synaptic protein expression (GluR1) and increased insertion and density of synapses – leading to increased structural connectivity between neurons, particularly in the pre-frontal cortex.

6. Summary

Apart from its well-known NMDA blockade, ketamine disturbs a wide range of intracellular neuronal processes. It seems likely that the hypnotic effects are caused by a combination of immediate channel blockade of NMDA and HCN1 channels. In contrast, the prolonged antidepressant effects probably arise from downstream "post-drug" effects – such as activity-induced increase in structural synaptic connectivity. The analgesic effects of ketamine appear to involve both short term and long term disturbance of cellular function. Probably its immediate analgesic effects are mediated predominantly by a combination of opioid system sensitisation and aminergic anti-nociception, whereas its inhibition of neuropathic pain relies on a combination of immediate receptor mediated action and initiation of longer lasting cell signalling cascades.

7. Outstanding questions

1) To what extent can the analgesic and the sedative components of ketamine be separated on the basis of their differing neuronal mechanisms?
2) Would inhibition of the ketamine-induced hyper-glutamatergic and hyper-aminergic states, improve or worsen the psychotomimesis, analgesia, and hypnosis?
3) How much would direct manipulation of the intracellular signalling cascades alter the clinical effects of ketamine?

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Conflict of interest

The authors are holders on a patent related to ketamine derivatives – number 602885.

References

1. Domino EF, Chodoff P, Corssen G. Pharmacologic effects of Ci-581, a new dissociative anesthetic, in man. *Clin Pharmacol Ther* 1965;**6**:279–91. Epub 1965/05/01.
2. MacDonald JF, Miljkovic Z, Pennefather P. Use-dependent block of excitatory amino acid currents in cultured neurons by ketamine. *J Neurophysiol* 1987;**58**(2):251–66. Epub 1987/08/01.
3. Hirota K. Special cases: ketamine, nitrous oxide and xenon. *Best Pract Res Clin Anaesthesiol* 2006;**20**(1):69–79. Epub 2006/04/26.
4. Duman RS, Li N, Liu RJ, Duric V, Aghajanian G. Signaling pathways underlying the rapid antidepressant actions of ketamine. *Neuropharmacology* 2012;**62**(1):35–41. Epub 2011/09/13.
5. Dorandeu F. Happy 50th anniversary ketamine. *CNS Neurosci Ther* 2013;**19**(6):369.
6. Cai YC, Ma L, Fan GH, Zhao J, Jiang LZ, Pei G. Activation of N-methyl-D-aspartate receptor attenuates acute responsiveness of delta-opioid receptors. *Mol Pharmacol* 1997;**51**(4):583–7. Epub 1997/04/01.
7. Lydic R, Baghdoyan HA. Ketamine and MK-801 decrease acetylcholine release in the pontine reticular formation, slow breathing, and disrupt sleep. *Sleep* 2002;**25**(6):617–22. Epub 2002/09/13.
8. Yamakura T, Chavez-Noriega LE, Harris RA. Subunit-dependent inhibition of human neuronal nicotinic acetylcholine receptors and other ligand-gated ion channels by dissociative anesthetics ketamine and dizocilpine. *Anesthesiology* 2000;**92**(4):1144–53. Epub 2001/02/07.
9. Kubota T, Anzawa N, Hirota K, Yoshida H, Kushikata T, Matsuki A. Effects of ketamine and pentobarbital on noradrenaline release from the medial prefrontal cortex in rats. *Can J Anaesth* 1999;**46**(4):388–92. Epub 1999/05/08.
10. Kamiyama H, Matsumoto M, Otani S, Kimura SI, Shimamura KI, Ishikawa S, et al. Mechanisms underlying ketamine-induced synaptic depression in rat hippocampus-medial prefrontal cortex pathway. *Neuroscience* 2011;**177**:159–69. Epub 2010/12/18.
11. Wang M, Wong AH, Liu F. Interactions between NMDA and dopamine receptors: a potential therapeutic target. *Brain Res* 2012;**1476**:154–63. Epub 2012/04/05.
12. Kussius CL, Kaur N, Popescu GK. Pregnanolone sulfate promotes desensitization of activated NMDA receptors. *J Neurosci* 2009;**29**(21):6819–27. Epub 2009/05/29.
13. Yamakage M, Hirshman CA, Croxton TL. Inhibitory effects of thiopental, ketamine, and propofol on voltage-dependent Ca2+ channels in porcine tracheal smooth muscle cells. *Anesthesiology* 1995;**83**(6):1274–82. Epub 1995/12/01.
14. Xue JG, Masuoka T, Gong XD, Chen KS, Yanagawa Y, Law SK, et al. NMDA receptor activation enhances inhibitory GABAergic transmission onto hippocampal pyramidal neurons via presynaptic and postsynaptic mechanisms. *J Neurophysiol* 2011;**105**(6):2897–906. Epub 2011/04/08.
15. Ogden KK, Traynelis SF. New advances in NMDA receptor pharmacology. *Trends Pharmacol Sci* 2011;**32**(12):726–33. Epub 2011/10/15.
16. Vance KM, Simorowski N, Traynelis SF, Furukawa H. Ligand-specific deactivation time course of GluN1/GluN2D NMDA receptors. *Nat Commun* 2011;**2**:294. Epub 2011/04/28.
17. Weigt HU, Adolph O, Georgieff M, Georgieff EM, Fohr KJ. Evidence that Xenon does not produce open channel blockade of the NMDA receptor. *J Neurophysiol* 2008;**99**(4):1983–7. Epub 2008/02/01.
18. Bolshakov KV, Gmiro VE, Tikhonov DB, Magazanik LG. Determinants of trapping block of N-methyl-d-aspartate receptor channels. *J Neurochem* 2003;**87**(1):56–65. Epub 2003/09/13.
19. Lanthorn TH, Mealing GA, Morley P. Differences in degree of trapping between AR-R15896 and other uncompetitive NMDA receptor antagonists. *Amino Acids* 2000;**19**(1):173–5. Epub 2000/10/12.
20. Lipton SA. Pathologically-activated therapeutics for neuroprotection: mechanism of NMDA receptor block by memantine and S-nitrosylation. *Curr Drug Targets* 2007;**8**(5):621–32. Epub 2007/05/17.
21. Belluardo N, Mudo G, Dell'Albani P, Jiang XH, Condorelli DF. NMDA receptor-dependent and -independent immediate early gene expression induced by focal mechanical brain injury. *Neurochem Int* 1995;**26**(5):443–53. Epub 1995/05/01.
22. Mei XP, Wang W, Wang W, Zhu C, Chen L, Zhang T, et al. Combining ketamine with astrocytic inhibitor as a potential analgesic strategy for neuropathic pain ketamine, astrocytic inhibitor and pain. *Mol Pain* 2010;**6**:50.
23. Ohnesorge H, Feng Z, Zitta K, Steinfath M, Albrecht M, Bein B. Influence of clonidine and ketamine on m-RNA expression in a model of opioid-induced hyperalgesia in mice. *PLoS One* 2013;**8**(11):e79567. Epub 2013/11/14.
24. Hayashi Y, Kawaji K, Sun L, Zhang X, Koyano K, Yokoyama T, et al. Microglial Ca(2+)-activated K(+) channels are possible molecular targets for the analgesic effects of S-ketamine on neuropathic pain. *J Neurosci* 2011;**31**(48):17370–82.
25. Mei X, Wang W, Wang W, Li Y, Zhang H, Wu S, et al. Inhibiting astrocytic activation: a novel analgesic mechanism of ketamine at the spinal level? *J Neurochem* 2009;**109**(6):1691–700.
26. Garcia LS, Comim CM, Valvassori SS, Reus GZ, Barbosa LM, Andreazza AC, et al. Acute administration of ketamine induces antidepressant-like effects in the forced swimming test and increases BDNF levels in the rat hippocampus. *Prog Neuropsychopharmacol Biol Psychiatry* 2008;**32**(1):140–4.
27. Yang C, Hu YM, Zhou ZQ, Zhang GF, Yang JJ. Acute administration of ketamine in rats increases hippocampal BDNF and mTOR levels during forced swimming test. *Ups J Med Sci* 2013;**118**(1):3–8.
28. Sorce S, Schiavone S, Tucci P, Colaianna M, Jaquet V, Cuomo V, et al. The NADPH oxidase NOX2 controls glutamate release: a novel mechanism involved in psychosis-like ketamine responses. *J Neurosci* 2010;**30**(34):11317–25. Epub 2010/08/27.
29. Stratinaki M, Varidaki A, Mitsi V, Ghose S, Magida J, Dias C, et al. Regulator of G protein signaling 4 [corrected] is a crucial modulator of antidepressant drug action in depression and neuropathic pain models. *Proc Natl Acad Sci U S A* 2013;**110**(20):8254–9. Epub 2013/05/01.
30. Clements JA, Nimmo WS, Grant IS. Bioavailability, pharmacokinetics, and analgesic activity of ketamine in humans. *J Pharm Sci* 1982;**71**(5):539–42. Epub 1982/05/01.
31. Mion G, Villevieille T. Ketamine pharmacology: an update (pharmacodynamics and molecular aspects, recent findings). *CNS Neurosci Ther* 2013;**19**(6):370–80.
32. Homayoun H, Moghaddam B. NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *J Neurosci* 2007;**27**(43):11496–500. Epub 2007/10/26.
33. Kelland MD, Soltis RP, Boldry RC, Walters JR. Behavioral and electrophysiological comparison of ketamine with dizocilpine in the rat. *Physiol Behav* 1993;**54**(3):547–54. Epub 1993/09/01.
34. Petrenko AB, Yamakura T, Fujiwara N, Askalany AR, Baba H, Sakimura K. Reduced sensitivity to ketamine and pentobarbital in mice lacking the N-methyl-D-aspartate receptor GluRepsilon1 subunit. *Anesth Analg* 2004;**99**(4):1136–40. Table of contents. Epub 2004/09/24.
35. Petrenko AB, Yamakura T, Kohno T, Sakimura K, Baba H. Increased brain monoaminergic tone after the NMDA receptor GluN2A subunit gene knockout is responsible for resistance to the hypnotic effect of nitrous oxide. *Eur J Pharmacol* 2013;**698**(1–3):200–5. Epub 2012/11/06.
36. Chen X, Shu S, Bayliss DA. HCN1 channel subunits are a molecular substrate for hypnotic actions of ketamine. *J Neurosci* 2009;**29**(3):600–9. Epub 2009/01/23.
37. Zhou C, Douglas JE, Kumar NN, Shu S, Bayliss DA, Chen X. Forebrain HCN1 channels contribute to hypnotic actions of ketamine. *Anesthesiology* 2013;**118**(4):785–95. Epub 2013/02/05.
38. Lu J, Nelson LE, Franks N, Maze M, Chamberlin NL, Saper CB. Role of endogenous sleep-wake and analgesic systems in anesthesia. *J Comp Neurol* 2008;**508**(4):648–62. Epub 2008/04/03.
39. Nelson CL, Burk JA, Bruno JP, Sarter M. Effects of acute and repeated systemic administration of ketamine on prefrontal acetylcholine release and sustained attention performance in rats. *Psychopharmacol (Berl)* 2002;**161**(2):168–79. Epub 2002/05/01.
40. Dahan A, Olofsen E, Sigtermans M, Noppers I, Niesters M, Aarts L, et al. Population pharmacokinetic-pharmacodynamic modeling of ketamine-induced pain relief of chronic pain. *Eur J Pain* 2011;**15**(3):258–67. Epub 2010/07/20.
41. Niesters M, Martini C, Dahan A. Ketamine for chronic pain: risks and benefits. *Br J Clin Pharmacol* 2014;**77**(2):357–67.
42. Rabben T, Oye I. Interindividual differences in the analgesic response to ketamine in chronic orofacial pain. *Eur J Pain* 2001;**5**(3):233–40. Epub 2001/09/18.
43. Olofsen E, Noppers I, Niesters M, Kharasch E, Aarts L, Sarton E, et al. Estimation of the contribution of norketamine to ketamine-induced acute pain relief and neurocognitive impairment in healthy volunteers. *Anesthesiology* 2012;**117**(2):353–64. Epub 2012/06/14.
44. Niesters M, Dahan A, Swartjes M, Noppers I, Fillingim RB, Aarts L, et al. Effect of ketamine on endogenous pain modulation in healthy volunteers. *Pain* 2011;**152**(3):656–63. Epub 2011/01/18.
45. Noppers I, Niesters M, Aarts L, Smith T, Sarton E, Dahan A. Ketamine for the treatment of chronic non-cancer pain. *Expert Opin Pharmacother* 2010;**11**(14):2417–29. Epub 2010/09/11.
46. Rabben T, Skjelbred P, Oye I. Prolonged analgesic effect of ketamine, an N-methyl-D-aspartate receptor inhibitor, in patients with chronic pain. *J Pharmacol Exp Ther* 1999;**289**(2):1060–6. Epub 1999/04/24.
47. Petrenko AB, Yamakura T, Baba H, Shimoji K. The role of N-methyl-D-aspartate (NMDA) receptors in pain: a review. *Anesth Analg* 2003;**97**(4):1108–16. Epub 2003/09/23.
48. Sarton E, Teppema LJ, Olievier C, Nieuwenhuijs D, Matthes HW, Kieffer BL, et al. The involvement of the mu-opioid receptor in ketamine-induced respiratory depression and antinociception. *Anesth Analg* 2001;**93**(6):1495–500. Table of contents. Epub 2001/12/01.
49. Shikanai H, Hiraide S, Kamiyama H, Kiya T, Oda K, Goto Y, et al. Subanalgesic ketamine enhances morphine-induced antinociceptive activity without cortical dysfunction in rats. *J Anesth*; 2013. Epub 2013/10/12.
50. Blednov YA, Stoffel M, Alva H, Harris RA. A pervasive mechanism for analgesia: activation of GIRK2 channels. *Proc Natl Acad Sci U S A* 2003;**100**(1):277–82. Epub 2002/12/21.
51. Gupta A, Devi LA, Gomes I. Potentiation of mu-opioid receptor-mediated signaling by ketamine. *J Neurochem* 2011;**119**(2):294–302. Epub 2011/06/23.
52. Koizuka S, Obata H, Sasaki M, Saito S, Goto F. Systemic ketamine inhibits hypersensitivity after surgery via descending inhibitory pathways in rats. *Can J Anaesth* 2005;**52**(5):498–505.
53. Gordh T, Karlsten R, Kristensen J. Intervention with spinal NMDA, adenosine, and NO systems for pain modulation. *Ann Med* 1995;**27**(2):229–34.
54. Backonja M, Arndt G, Gombar KA, Check B, Zimmermann M. Response of chronic neuropathic pain syndromes to ketamine: a preliminary study. *Pain* 1994;**56**:51–7.
55. Warncke T, Stubhaug A, Jorum E. Ketamine, an NMDA receptor antagonist, suppresses spatial and temporal properties of burn-induced secondary

hyperalgesia in man: a double-blind, cross-over comparison with morphine and placebo. *Pain* 1997;**72**(1–2):99–106. Epub 1997/08/01.
56. Remerand F, Le Tendre C, Baud A, Couvret C, Pourrat X, Favard L, et al. The early and delayed analgesic effects of ketamine after total hip arthroplasty: a prospective, randomized, controlled, double-blind study. *Anesth Analg* 2009;**109**(6):1963–71. Epub 2009/11/20.
57. Romero-Sandoval EA. Depression and pain: does ketamine improve the quality of life of patients in chronic pain by targeting their mood? *Anesthesiology* 2011;**115**(4):687–8. Epub 2011/08/16.
58. Wang J, Goffer Y, Xu D, Tukey DS, Shamir DB, Eberle SE, et al. A single subanesthetic dose of ketamine relieves depression-like behaviors induced by neuropathic pain in rats. *Anesthesiology* 2011;**115**(4):812–21. Epub 2011/09/22.
59. Geisser ME, Roth RS, Theisen ME, Robinson ME, Riley 3rd JL. Negative affect, self-report of depressive symptoms, and clinical depression: relation to the experience of chronic pain. *Clin J Pain* 2000;**16**(2):110–20. Epub 2000/06/28.
60. Zimmermann M. Pathobiology of neuropathic pain. *Eur J Pharmacol* 2001;**429**(1–3):23–37.
61. Liu RJ, Lee FS, Li XY, Bambico F, Duman RS, Aghajanian GK. Brain-derived neurotrophic factor Val66Met allele impairs basal and ketamine-stimulated synaptogenesis in prefrontal cortex. *Biol Psychiatry* 2012;**71**(11):996–1005. Epub 2011/11/01.