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Ketamine Intoxication in Two Species of Non-Human Primates

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14. ABSTRACT
This report summarizes casual observations collected during routine veterinary care and provides preliminary information on overt toxicity and incapacitation following intramuscular injection of ketamine in two species of non-human primates: African green vervet monkeys (*Chlorocebus aethiops sabeus*) and cynomolgus macaques (*Macaca fascicularis*). The onset of ataxia and immobilization were recorded after a 10 mg/kg dose of ketamine for both species, and also for a 5 mg/kg dose for the African green monkeys. For all data sets, both sexes were represented, and despite the substantially lower body weights in females, no apparent sex differences were detected. Rapid onset of ataxia (in less than 3 minutes) and immobilization (in less than 5 minutes) was observed in all cases, regardless of species. These data are limited by the observational methods used and the limited range of doses and routes of exposure, but the conclusions are bolstered by a wealth of data from this and other studies using ketamine for sedation/anesthesia in non-human primates. This study provides important preliminary information on the rapid onset of ataxia and immobilization in males and females of two common species of laboratory non-human primates and offers a starting point for more focused investigations using comprehensive operant behavioral testing under an expanded range of ketamine doses and exposure routes. Such work would elaborate ketamine's intoxication profile and set the stage for discovering effective medical countermeasures for ketamine intoxication in these non-human primate models.

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Ketamine, African green monkey, cynomolgus macaques, intoxication, ataxia, immobilization, medical countermeasures

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ABSTRACT

This report summarizes casual observations collected during routine veterinary care and provides preliminary information on overt toxicity and incapacitation following intramuscular injection of ketamine in two species of non-human primates: African green vervet monkeys (*Chlorocebus aethiops sabeus*) and cynomolgus macaques (*Macaca fascicularis*). The onset of ataxia and immobilization were recorded after a 10 mg/kg dose of ketamine for both species, and also for a 5 mg/kg dose for the African green monkeys. For all data sets, both sexes were represented, and despite the substantially lower body weights in females, no apparent sex differences were detected. Rapid onset of ataxia (in less than 3 minutes) and immobilization (in less than 5 minutes) was observed in all cases, regardless of species. These data are limited by the observational methods used and the limited range of doses and routes of exposure, but the conclusions are bolstered by a wealth of data from this and other studies using ketamine for sedation/anesthesia in non-human primates. This study provides important preliminary information on the rapid onset of ataxia and immobilization in males and females of two common species of laboratory non-human primates and offers a starting point for more focused investigations using comprehensive operant behavioral testing under an expanded range of ketamine doses and exposure routes. Such work would elaborate ketamine's intoxication profile and set the stage for discovering effective medical countermeasures for ketamine intoxication in these non-human primate models.

INTRODUCTION

Ketamine (KET; Cl-581) is an arylcyclohexylamine derivative developed on the backbone of phenyl-cyclohexyl-piperidine (phencyclidine; commonly known as PCP) as an alternative to the then-popular anesthetic with a lower incidence of psychotomimetic side effects. KET is classified as a dissociative anesthetic because it produces a functional dissociation between the limbic and thalamocortical systems. While notably an N-methyl-D-aspartate (NMDA) receptor antagonist, KET has been shown to be active at a wide variety of receptors and receptor subtypes including, but not limited to, μ, δ, and κ opioid receptors, sigma receptors, and nicotinic and muscarinic cholinergic receptors, as well as to inhibit serotonin uptake and interact with acetylcholinesterase [1-6]. An active metabolite of KET, (2R,6R)-hydroxynorketamine, has also been shown to activate α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors [7]. KET, in its hydrochloride salt form, is highly water-soluble and can be administered intravenously, intramuscularly (IM), subcutaneously, rectally, sublingually, orally, transdermally and intranasally, as well as through a nebulized aerosol solution [8-11]. KET is rapidly and widely distributed following intravenous administration and follows a two-compartment model of elimination, with α and β phase half-lives of 11-17 minutes and 151-186, respectively [12]. KET is typically found in the form of a racemate hydrochloride salt, with both the R(-) and S(+) optical isomers present in equal amounts. In human subjects, the S(+) enantiomer induces more EEG suppression than the R(-) equivalent dose, with the R(-) enantiomer being unable to achieve the same level of EEG suppression as S(+) at higher doses due to a ceiling effect [13]. Similarly, the S(+) enantiomer has considerably more potency as a hypnotic and analgesic, as well as when inducing anesthesia (which R(-) often fails to achieve altogether) [14]. Recovery from the anesthetic effects of KET is shortest when S(+) is used alone rather than when R(-) or the racemate is used, suggesting an additive effect between the two enantiomers when administered together, possibly due to R(-) inhibiting the elimination of the more active S(+) enantiomer [13, 15]. The only clinical benefit of R(-) KET when used alone is that it may be a more potent and long-lasting antidepressant, perhaps owing to its reduced psychotomimetic effects [16]. KET has most often been studied in its racemic form, as was the case here (using the commonly available veterinary formulation).

KET's use in humans dates back to the mid-1960s when it was shown to be an effective fast- and short-acting anesthetic when given intravenously [17]. More recently it has been shown to have both analgesic [2, 18] and antidepressant [7, 19] effects at sub-anesthetic blood concentrations, which has broadened its use in clinical settings. Further, it has been shown to be an effective drug adjunct for treating numerous types of status epilepticus (SE), including refractory SE [20-23]. In some areas, KET is being utilized at supra-anesthetic doses for the rapid and prolonged sedation of acutely agitated patients in emergency medical settings via large-volume IM injection [24, 25]. KET has also been shown to be a safe and effective sole anesthetic agent in human clinical settings across a wide variety of doses during major surgical procedures such as open-heart surgeries, open-chest pulmonary surgeries, long-bone fracture repairs, and skin grafts [26-29]. In most cases of KET use as a sole anesthetic agent in humans, pre- or co-

medication with drugs such as atropine, diazepam, and/or various paralytic agents occurred. Fatal KET overdoses, although rare due to KET's high therapeutic index, mostly occur in the context of recreational drug abuse and often involve other factors such as underlying illness/injury and poly-drug abuse [30-33].

KET has been evaluated for use as a safe and rapid incapacitating agent in numerous large animal species (such as lions, chimpanzees, orangutans, horses, badgers, giraffes and otters, among others), both as a sole chemical agent [34-36] and in combination with other drugs, such as the phenothiazine drug promazine, the opioid butorphanol, and the α2-adrenergic antagonists xylazine and medetomidine (including various derivatives) [37-42]. When used as a sole incapacitating agent in badgers, KET doses ranged between 9.5 and 31.0 mg/kg IM (including repeated dosing), and incapacitation times ranged from 2-7 minutes post-injection. When KET was used in elephant seals (500-675 kg), IM doses ranged from 1.4 to 6.9 mg/kg, and incapacitation times ranged from 2-25 minutes [35, 36]. KET has long been used as an anesthetic for laboratory non-human primates (NHPs) due to its fast onset, short duration and wide margin of safety. Intramuscular administration has been shown to be an effective route of administration in laboratory NHPs compared to other routes (such as oral) due to KET's rapid onset and more predictable dose-response [43]. A KET dose of 10 mg/kg is a common anesthetic dose for NHPs, allowing a sufficient duration of immobilization to safely complete veterinary screenings or minor procedures such as dental work and blood collection. KET alone provides rapid incapacitation and a shorter duration of anesthesia than combinations of lower dose KET and reversible α2-adrenergic agents such as medetomidine, though the depth of anesthesia is lower with KET alone [44, 45]. Repeated daily dosing of a 10 mg/kg anesthetic dose of KET has been shown to cause statistically significant, though not necessarily dangerous, hematological changes in NHPs [46].

The data reported herein were not part of a formal study, but merely a collation of observations taken during routine veterinary care and examination of laboratory NHPs. Drug doses were those used previously for routine veterinary care, and were selected to be appropriate for temporary anesthesia and well below the potentially lethal range. Thus, the data presented here are not intended to comprise a comprehensive study of ketamine safety or its immobilization profile. Nevertheless, the data are of value and provide a strong reference point from which more focused studies may be initiated.

METHODS

Subjects

All monkeys had extensive laboratory experience and were adults between 5 and 12 years of age. Eleven female (3.70-4.80 kg, mean 4.10 kg) and twenty-two male (4.16-6.36, kg, mean 5.25 kg) African green monkeys (AGMs; *Chlorocebus aethiops sabeus*) of Caribbean origin experienced the 5 mg/kg KET dosing condition. A separate set of five female (3.90-4.54 kg, mean 4.20 kg) and seven male (5.76-6.48 kg, mean 6.12 kg) African green monkeys of identical

origin experienced the 10 mg/kg KET dosing condition. Cynomolgus macaques (*Macaca fascicularis*) of Asian origin (Cambodia, Vietnam) were included in a comparable 10 mg/kg KET condition, with four females (5.36-6.28 kg, mean 5.73 kg) and five males (6.52-7.58 kg, mean 7.14 kg). All animals were individually housed in stainless steel squeeze-back one-over-one NHP housing units (Allentown, LLC; Allentown, NJ) that could be interconnected laterally to additional housing units of identical configuration. Each unit consisted of two vertically stacked cages, each equipped with an automated watering system and an effective area of 82 cm W X 82 cm D X 85 cm H. The colony was maintained at 21 ± 2 °C with a relative humidity of 50% ± 15% on a 12 h light/dark cycle (lights on at 0600). Allotted food (Certified Primate Diet 5048, Purina Mills, Inc., St. Louis, MO), provided via cage-top feeding and supplemented daily with fresh fruit and vegetables, was controlled to maintain healthy body weights. Water was available *ad libitum*. Animals had continual visual and auditory access to conspecifics and were provided with a variety of enrichment devices (e.g., mirrors, chew toys, manipulanda, foraging boards). During operant behavioral testing (approximately 3 hours daily), animals were restricted to the lower half of the two-cage housing unit. On behavioral training and testing days, the food ration was provided at least one hour after testing concluded. The USAMRICD is a research facility fully accredited by AAALAC, International.

Chemicals and Procedure

Undiluted KET (Ketaset©, 100 mg/ml) was obtained from Zoetis (Parsippany, NJ) and injected IM into the anteriorlateral thigh muscle (or interiorlateral thigh in the case of one animal) using a 1 mL syringe affixed to a 27-gauge, ¾ inch needle.

Observations

KET was used for brief anesthesia as part of scheduled veterinary procedures, namely, periodic health examinations. Immediately after IM KET injection, each monkey was observed for onset of ataxia (uncoordinated movement, loss of balance), followed by the onset of immobilization (collapse, prostration), and the time from injection to onset of each was recorded.

Data Analysis

Statistical analyses were not warranted based on the lack of random assignment of subjects, lack of replication, and the limited range of ketamine doses examined. Descriptive (non-inferential) statistics (means and ranges) were computed using GraphPad Prism 5 for Windows (Version 5.04; GraphPad Software, San Diego, CA). Data are reported below for body weights, and the onset of ataxia and immobilization, as a function of species and sex. Graphically, the mean and corresponding 95% confidence intervals are included simply to aid visual analysis.

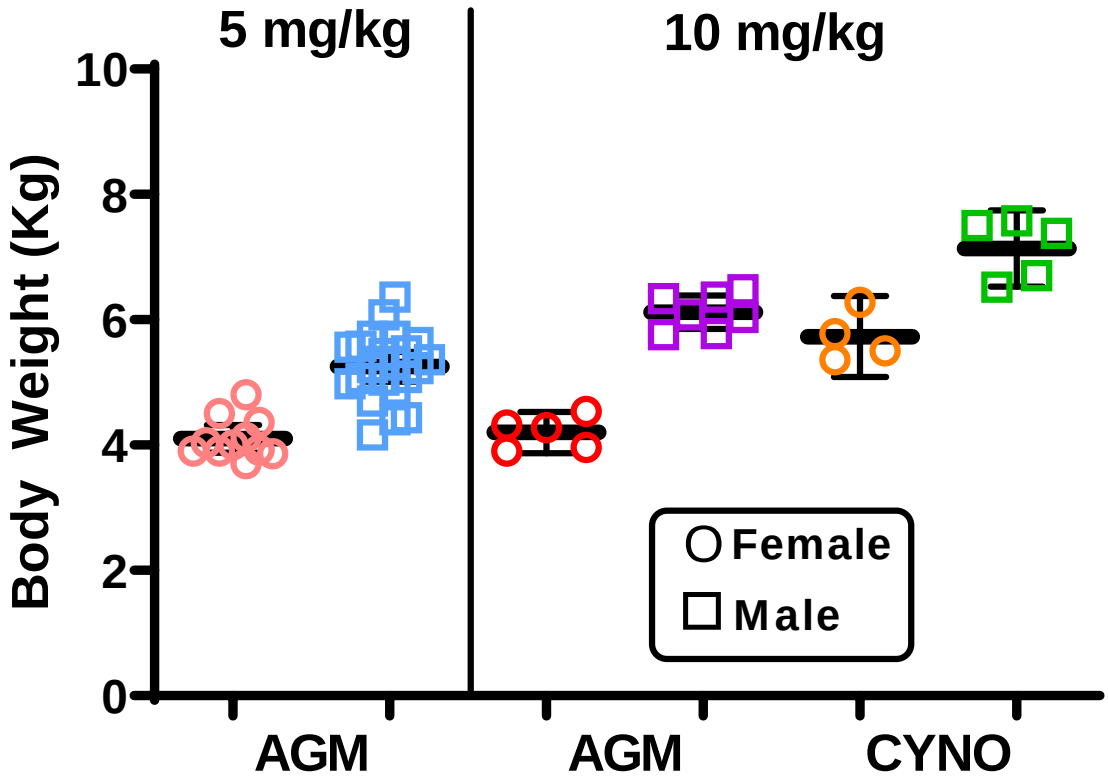

Figure 1. Body weights at the time of KET administration as a function of dose, species, and sex.

RESULTS

Figure 1 shows the individual body weights (along with the group mean and 95% confidence intervals) at the time of KET administration. Overall, female AGMs averaged 4.15 kg and weighed less than their male counterparts, although individual body weights of females and males overlapped in the 5 mg/kg dose group. A sex difference in mean body weight was more pronounced for AGMs in the 10 mg/kg group, wherein the males approximated 6.1 kg (compared to 5.3 kg in the 5 mg/kg dose group) and no overlap between sexes was observed. Female cynomolgus macaques averaged 5.73 kg and clearly weighed more than the female African green monkeys. Male cynomolgus macaques were heavier still, with an average weight of 7.13 kg and no overlap in individual weights observed with their female counterparts.

The main aim of this report was to index onset of ataxia and immobilization produced by KET following IM injection. Figure 2 shows the latency between administration of KET and the onset of ataxia, defined as wobbling, uncoordinated movement, and/or frank tremor. In the left panel of the figure are results for the 5 mg/kg dose, conducted only in the AGMs. Despite the lower dose, onset of ataxia was 5 min or less in all cases, with a mean (and mode) approximating 2 min in both sexes. Latencies for ataxia ranged from 1 to 4 min in females and 1 to 5 min in males. Thus, even at this lower dose, onset of ataxia was rapid and did not markedly differ

between sexes, with some inter-subject variability observed. In the 10 mg/kg condition in this same species, variability was reduced, with all subjects exhibiting ataxia within 1 to 3 min. A dose-dependent decrease was evident in the male, but not the female, AGMs. Nevertheless, mean onset of ataxia still approximated 2 min in both sexes. For the cynomolgus macaques, the 10 mg/kg dose produced a comparably rapid onset of ataxia, equaling 1 min for all four females studied and ranging from 1 to 2 min for the males. No obvious differences in response were observed as a function of species or sex, and the onset of ataxia induced by IM KET occurred within a few minutes.

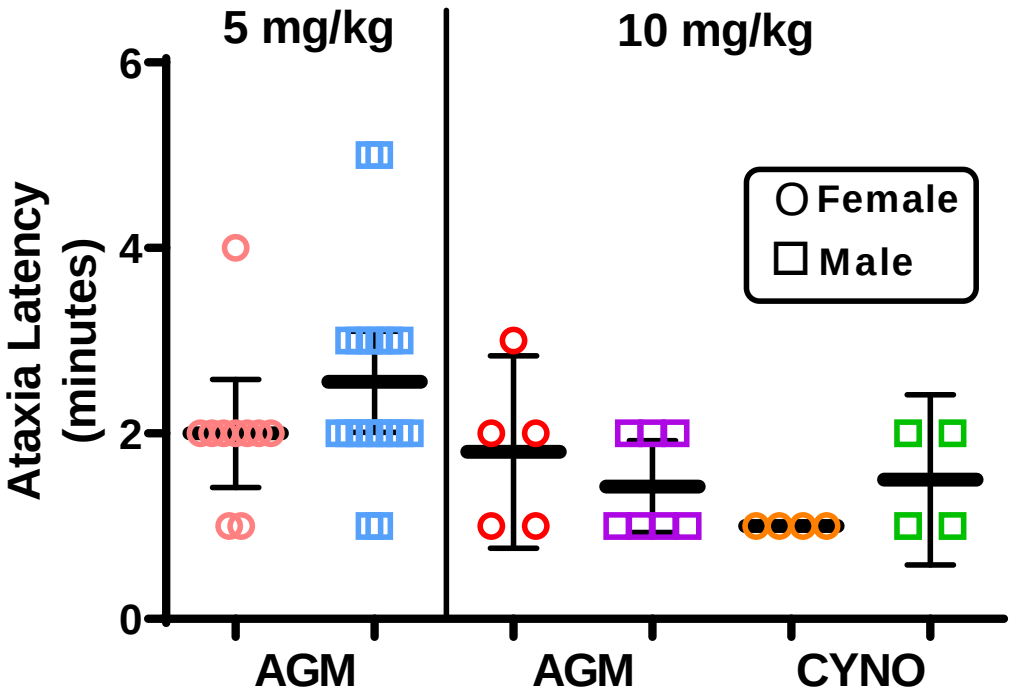

Figure 2. Latency to onset of ataxia (in minutes) as a function of dose (5 mg/kg or 10 mg/kg IM), species (African green monkey or cynomolgus macaque), and sex (Female or Male).

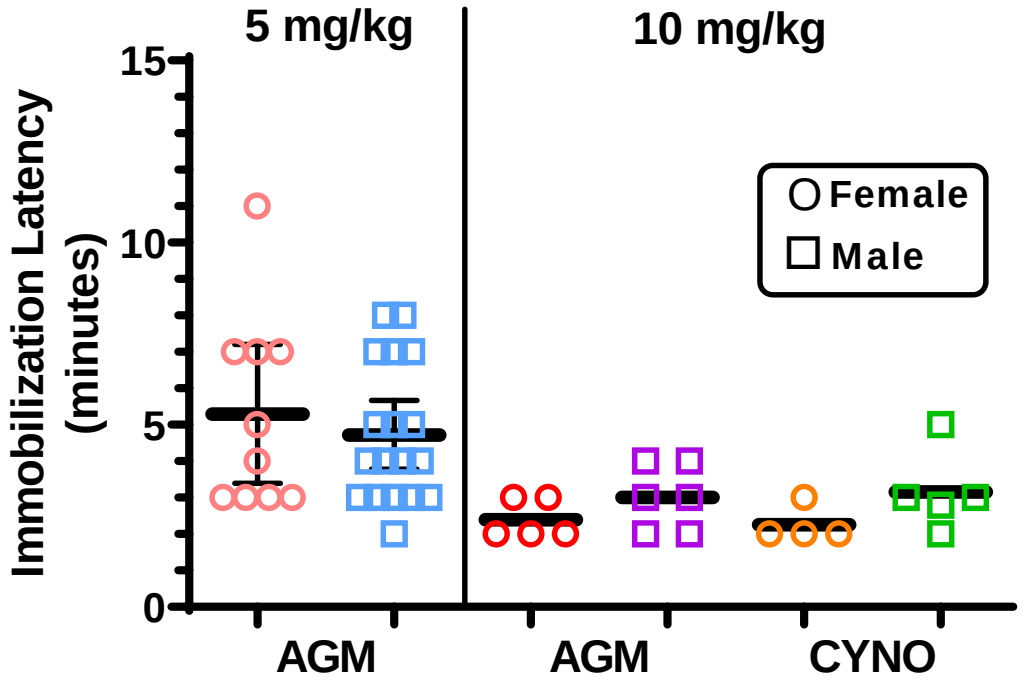

Figure 3. Latency to onset of immobilization (in minutes) as a function of dose (5 mg/kg or 10 mg/kg IM), species (African green monkey or cynomolgus macaque), and sex (Female or Male).

Figure 3 shows latency to immobilization, defined as reaching a non-surgical plane of anesthesia and sustained absence of movement (except breathing, palpebral, laryngeal, and withdrawal reflexes) for one full minute or more. At the 5 mg/kg dose, AGMs exhibited complete immobilization in under 11 min, sometimes as quickly as 2 min, and with an average of about 5 min in both females and males. The variability in immobilization latency was reduced at the higher KET dose of 10 mg/kg, with an average latency of 2.7 min across the AGMs and cynomolgus macaques of both sexes. Immobilization occurred within 5 min in all cases, demonstrating a rapid and profound intoxication by KET. As before, no obvious species or sex differences in response were discernible.

DISCUSSION

The onset and duration of KET-induced ataxia and immobilization following IM injection in two species of common laboratory primates, and both sexes, were observed during routine veterinary care. Both the 5 and 10 mg/kg doses produced very rapid ataxia and immobilization, and no apparent species or sex differences were observed. A trend toward more rapid and less variable response was observed at the lower KET dose (evaluated only in AGMs), suggesting a dose-dependent onset of intoxication. A mild (ataxia) to severe (immobilization) state of intoxication could be of considerable operational relevance following intentional or unintentional exposure to KET. In fact, much lower KET doses would likely produce profound

cognitive-behavioral impairments, more subtle than the frank intoxication evaluated herein. Also, the duration of intoxication was not evaluated in the present study, and much work remains to fully characterize KET from an operational perspective. In a related vein, because KET has no known antidotes, overdose with KET remains a concern.

Functional operant behavioral assessments have been successfully implemented many times in these NHP species, and such behavioral assessments would be a welcome addition and logical next step in characterizing KET toxicity. A more systematic dose-effect function and the inclusion of different exposure routes (such as inhalation), along with an emphasis on duration of intoxication and task-specific impairments (e.g., memory function, time estimation, fine motor control, speed discrimination, reaction time) should be evaluated [47-58]. Not only would this work better characterize KET intoxication, but it would set the stage for the development, testing, and optimization of medical treatment strategies for KET overdose.

REFERENCES

1. Durieux, M.E., Inhibition by ketamine of muscarinic acetylcholine receptor function. *Anesthesia & Analgesia*, 1995. **81**(1): p. 57-62.
2. Persson, J., Ketamine in pain management. *CNS Neuroscience & Therapeutics*, 2013. **19**(6): p. 396-402.
3. Domino, E.F., Taming the ketamine tiger. *Anesthesiology: The Journal of the American Society of Anesthesiologists*, 2010. **113**(3): p. 678-684.
4. Schneider, P.G. and G. Rodriguez de Lores Arnaiz, Ketamine prevents seizures and reverses changes in muscarinic receptor induced by bicuculline in rats. *Neurochemistry International*, 2013. **62**(3): p. 258-64.
5. Martin, L. and D. Smith, Ketamine inhibits serotonin synthesis and metabolism in vivo. *Neuropharmacology*, 1982. **21**(2): p. 119-125.
6. Puu, G., M. Koch, and E. Artursson, Ketamine enantiomers and acetylcholinesterase. *Biochemical Pharmacology*, 1991. **41**(12): p. 2043-5.
7. Zanos, P., et al., NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature*, 2016. **533**(7604): p. 481.
8. Kronenberg, R.H., Ketamine as an analgesic: parenteral, oral, rectal, subcutaneous, transdermal and intranasal administration. *Journal of Pain & Palliative Care Pharmacotherapy*, 2002. **16**(3): p. 27-35.
9. Donnelly, R.F., Stability of diluted ketamine packaged in glass vials. *Canadian Journal of Hospital Pharmacy*, 2013. 66(3).
10. Chong, C., et al., Development of a sublingual/oral formulation of ketamine for use in neuropathic pain. *Clinical Drug Investigation*, 2009. **29**(5): p. 317-324.
11. Zhu, M.M., et al., Effects of nebulized ketamine on allergen-induced airway hyperresponsiveness and inflammation in actively sensitized Brown-Norway rats. *Journal of Inflammation*, 2007. **4**(1): p. 10.
12. Clements, J. and W. Nimmo, Pharmacokinetics and analgesic effect of ketamine in man. *BJA: British Journal of Anaesthesia*, 1981. **53**(1): p. 27-30.
13. White, P., et al., Comparative pharmacology of the ketamine isomers: studies in volunteers. *BJA: British Journal of Anaesthesia*, 1985. **57**(2): p. 197-203.
14. White, P.F., et al., Pharmacology of ketamine isomers in surgical patients. *Anesthesiology: The Journal of the American Society of Anesthesiologists*, 1980. **52**(3): p. 231-239.
15. Ihmsen, H., G. Geisslinger, and J. Schüttler, Stereoselective pharmacokinetics of ketamine: R (−)-Ketamine inhibits the elimination of S (+)-ketamine. *Clinical Pharmacology & Therapeutics*, 2001. **70**(5): p. 431-438.
16. Zhang, J.-c., S.-x. Li, and K. Hashimoto, R (−)-ketamine shows greater potency and longer lasting antidepressant effects than S (+)-ketamine. *Pharmacology Biochemistry and Behavior*, 2014. **116**: p. 137-141.

17. Domino, E.F., P. Chodoff, and G. Corssen, Pharmacologic effects of CI-581, a new dissociative anesthetic, in man. *Clinical Pharmacology & Therapeutics*, 1965. **6**(3): p. 279-291.
18. Laskowski, K., et al., A systematic review of intravenous ketamine for postoperative analgesia. *Canadian Journal of Anesthesia/Journal Canadien d'Anesthésie*, 2011. **58**(10): p. 911.
19. Monteggia, L.M. and C. Zarate Jr, Antidepressant actions of ketamine: from molecular mechanisms to clinical practice. *Current Opinion in Neurobiology*, 2015. **30**: p. 139-143.
20. Niquet, J., et al., Midazolam-ketamine dual therapy stops cholinergic status epilepticus and reduces Morris water maze deficits. *Epilepsia*, 2016. **57**(9): p. 1406-15.
21. Zeiler, F.A., Early use of the NMDA receptor antagonist ketamine in refractory and superrefractory status epilepticus. *Critical Care Research Practice*, 2015. **2015**: p. 831260.
22. Fang, Y. and X. Wang, Ketamine for the treatment of refractory status epilepticus. *Seizure*, 2015. **30**: p. 14-20.
23. Synowiec, A.S., et al., Ketamine use in the treatment of refractory status epilepticus. *Epilepsy Research*, 2013. **105**(1-2): p. 183-8.
24. Hopper, A.B., et al., Ketamine use for acute agitation in the emergency department. *The Journal of Emergency Medicine*, 2015. **48**(6): p. 712-719.
25. Scheppke, K.A., et al., Prehospital use of im ketamine for sedation of violent and agitated patients. *Western Journal of Emergency Medicine*, 2014. **15**(7): p. 736.
26. Corssen, G., et al., Ketamine as the sole anesthetic in open-heart surgery: a preliminary report. *Anesthesia & Analgesia*, 1970. **49**(6): p. 1025-1031.
27. Stefansson, T., I. Wickstrom, and H. Haijamäe, Hemodynamic and metabolic effects of ketamine anesthesia in the geriatric patient. *Acta Anaesthesiologica Scandinavica*, 1982. **26**(4): p. 371-377.
28. Rees, D.I. and M.L. Howell, Ketamine-atracurium by continuous infusion as the sole anesthetic for pulmonary surgery. *Anesthesia and Analgesia*, 1986. **65**(8): p. 860-864.
29. Garfield, J.M., M. MA, and F.B. Garfield, A comparison of psychologic responses to ketamine and thiopental---nitrous ovide—halothane anesthesia. *Anesthesiology*, 1972. **36**(4): p. 329-338.
30. Lalonde, B.R. and H.R. Wallage, Postmortem blood ketamine distribution in two fatalities. *Journal of Analytical Toxicology*, 2004. **28**(1): p. 71-74.
31. Peyton, S.H., A.T. Couch, and R.O. Bost, Tissue distribution of ketamine: two case reports. *Journal of Analytical Toxicology*, 1988. **12**(5): p. 268-269.
32. Schifano, F., et al., Trapped in the" K-hole": overview of deaths associated with ketamine misuse in the UK (1993-2006). *Journal of Clinical Psychopharmacology*, 2008. **28**(1): p. 114-116.
33. Gill, J. and M. Stajic, Ketamine in non-hospital and hospital deaths in New York City. *Journal of Forensic Science*, 2000. **45**(3): p. 655-658.

34. Smuts, G., et al., Some practical advantages of CI-581 (ketamine) for the field immobilization of larger wild felines, with comparative notes on baboons and impala. *The Lammgeyer*, 1973. **18**: p. 1-14.
35. Briggs, G.D., R.V. Henrickson, and B.J. Le Boeuf, Ketamine immobilization of northern elephant seals. *Journal of the American Veterinary Medical Association*, 1975. **167**(7): p. 546-548..
36. Mackintosh, C., et al., The immobilization of the badger (Meles meles). *British Veterinary Journal*, 1976. **132**(6): p. 609-614.
37. Herbst, L.H., C. Packer, and U. Seal, Immobilization of free-ranging African lions (Panthera leo) with a combination of xylazine hydrochloride and ketamine hydrochloride. *Journal of Wildlife Diseases*, 1985. **21**(4): p. 401-404.
38. Hess, L., et al., Experience with a naphthylmedetomidine–ketamine–hyaluronidase combination in inducing immobilization in anthropoid apes. *Journal of Medical Primatology*, 2010. **39**(3): p. 151-159.
39. Fuentes, V., Short term immobilization in the horse with ketamine HCl and promazine HCl combinations. *Equine Veterinary Journal*, 1978. **10**(2): p. 78-81.
40. McLaren, G.W., et al., The use and assessment of ketamine–medetomidine–butorphanol combinations for field anaesthesia in wild European badgers (Meles meles). *Veterinary Anaesthesia and Analgesia*, 2005. **32**(6): p. 367-372.
41. Bush, M., et al., Use of medetomidine and ketamine for immobilization of free-ranging giraffes. *Journal of the American Veterinary Medical Association*, 2001. **218**(2): p. 245-249.
42. Fernandez-Moran, J., et al., Reversible immobilization of Eurasian otters with a combination of ketamine and medetomidine. *Journal of Wildlife Diseases*, 2001. **37**(3): p. 561-565.
43. Winterborn, A.N., et al., The efficacy of orally dosed ketamine and ketamine/medetomidine compared with intramuscular ketamine in rhesus macaques (Macaca mulatta) and the effects of dosing route on haematological stress markers. *Journal of Medical Primatology*, 2008. **37**(3): p. 116-127.
44. Sun, F.J., D.E. Wright, and D.M. Pinson, Comparison of ketamine versus combination of ketamine and medetomidine in injectable anesthetic protocols: chemical immobilization in macaques and tissue reaction in rats. *Journal of the American Association for Laboratory Animal Science*, 2003. **42**(4): p. 32-37.
45. Lee, V.K., et al., Comparison of the effects of ketamine, ketamine–medetomidine, and ketamine–midazolam on physiologic parameters and anesthesia-induced stress in rhesus (Macaca mulatta) and cynomolgus (Macaca fascicularis) macaques. *Journal of the American Association for Laboratory Animal Science*, 2010. **49**(1): p. 57-63.
46. Lugo-Roman, L., et al., Effects of serial anesthesia using ketamine or ketamine/medetomidine on hematology and serum biochemistry values in rhesus macaques (Macaca mulatta). *Journal of Medical Primatology*, 2010. **39**(1): p. 41-49.

47. Myers, T.M. and M.G. Clark, Serial-probe recognition in rhesus macaques: effects of midazolam. *Pharmacology Biochemistry and Behavior*, 2006. **85**(3): p. 555-61.
48. Myers, T.M., et al., Effects of anticholinergics on serial-probe recognition accuracy of rhesus macaques (Macaca mulatta). *Pharmacology Biochemistry and Behavior*, 2002. **73**(4): p. 829-34.
49. Myers, T.M. and L.R. Hamilton, Delayed match-to-sample performance in African green monkeys (Chlorocebus aethiops sabaeus): effects of benzodiazepine, cholinergic, and anticholinergic drugs. *Behavioral Pharmacology*, 2011. **22**(8): p. 814-23.
50. Rice, N.C., J.R. Makar, and T.M. Myers, Sex and the stimulus-movement effect: Differences in acquisition of autoshaped responding in cynomolgus monkeys. *Physiology & Behavior*, 2017. **171**: p. 40-49.
51. Hamilton, L.R., D.M. Cox, and T.M. Myers, Use of operant performance to guide and evaluate medical treatment in an adult male cynomolgus macaque (Macaca fascicularis). *Journal of the American Association for Laboratory Animal Science*, 2011. **50**(6): p. 946-948.
52. Myers, T.M., Human plasma-derived butyrylcholinesterase is behaviorally safe and effective in cynomolgus macaques (Macaca fascicularis) challenged with soman. *Chemico-Biological Interactions*, 2019. **308**: p. 170-178.
53. Myers, T.M., et al., Systemic administration of the potential countermeasure huperzine reversibly inhibits central and peripheral acetylcholinesterase activity without adverse cognitive–behavioral effects. *Pharmacology Biochemistry and Behavior*, 2010. **94**(3): p. 477-481.
54. Hamilton, L.R. and T.M. Myers, Bioscavengers to protect against chemical warfare nerve agents: safety of adenovirus-driven expression of butyrylcholinesterase in macaques. *The Federation of American Societies for Experimental Biology (FASEB) Journal*, 2013 **27**(1_supplement), 662.8
55. Myers, T.M., et al., Characterization of human serum butyrylcholinesterase in rhesus monkeys: behavioral and physiological effects. *Neurotoxicology and Teratology*, 2012. **34**(3): p. 323-330.
56. Myers, T.M., et al., Huperzine A: *Behavioral and Pharmacological Evaluation in Rhesus Monkeys*. USAMRICD-TR-08-05. 2008, Aberdeen Proving Ground, MD: US Army Medical Research Institute of Chemical Defense. A501469
57. Langston, J.L., et al., Carfentanil toxicity in the African green monkey: therapeutic efficacy of naloxone. *Toxicology Letters*, 2020. **325**: 34-42.
58. Moffett, M.C., et al., Behavioral, pharmacokinetic, and cardiovascular evaluation of candidate oximes in African green monkeys. *International Journal of Toxicology*, 2018. **37**(5): p. 352-363.