Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Chronic morphine drinking establishes morphine tolerance, but not addiction in Wistar rats*

BINSACK Ralf[1], ZHENG Ming-lan[1,2], ZHANG Zhan-sai[1], YANG Liu[1], ZHU Yong-ping[†‡1]

([1]*Department of Toxicology, School of Medicine, Zhejiang University, Hangzhou 310006, China*)

([2]*Zhejiang Center for Disease Prevention and Control, Hangzhou 310009, China*)

[†]E-mail: zhuyp@zju.edu.cn

Received Mar. 16, 2006; revision accepted July 31, 2006

Abstract: Objective: Some animal models apply morphine in the drinking water to generate addiction, but related reports are not free of conflicting results. Accordingly, this study aimed to figure out if chronic consumption of morphine in the drinking water can induce morphine addiction in Wistar rats. Methods: For 3 weeks, the animals received a daily morphine dose of 35 mg/kg by offering a calculated volume of sugar water (5% sucrose) with morphine (0.1 mg/ml) to each rat; animals receiving just sugar water served as controls. Immediately after the treatment phase, the tail immersion test was used to check for morphine tolerance, and all animals were then kept on tap water for one week (withdrawal phase). Afterwards, all rats were allowed to choose their drinking source by offering two bottles, containing sugar water without and with morphine, simultaneously for two days (preference phase). Results: While the chronic consumption of morphine led to a reduction in body weight and to morphine tolerance, the morphine-treated Wistar rats did not show any preference for the opiate-containing sugar water. Conclusion: Body weight loss and tolerance do not reveal a condition of drug craving, and current animal models should be re-evaluated regarding their potential to establish morphine addicted animals.

Key words: Addiction, Morphine, Self-administration, Tolerance, Wistar rats
doi:10.1631/jzus.2006.B0892 **Document code:** A **CLC number:** Q42; R99

INTRODUCTION

According to the World Health Organization, more than 2 billion people are currently using alcohol, tobacco as well as a variety of illicit drugs (e.g. cannabis, cocaine, heroin) (Monteiro, 2001; WHO, 2002). These components are also known as "psychoactive substances" as they alter feelings, thinking and behavior, and the frequent use of those stimulants often leads to addiction, which is a chronically relapsing disorder characterized by a compulsive need for the drug, by recognizable physiological changes in the absence of the drug, and by an uncontrolled intake of the drug (Cami and Farre, 2003; Koob *et al*., 1998). Besides the loss of control, drug abuse in the form of an enhanced intake is also due to the development of tolerance, demanding the consumption of higher doses to experience the same stimulating effect. An addiction to drugs has been recognized as a neurological disease, and current research efforts focus on a better understanding of the involved molecular, cellular and physiological mechanisms (Ammon-Treiber and Hollt, 2005; Bailey and Connor, 2005; Koob *et al*., 2004). Besides taking measures to prevent a permanent intake of drugs, a better understanding of the phenomenon of addiction might also help to establish effective therapies for drug addicts, improving either the detoxification process or preventing the relapse (van den Brink and van Ree, 2003).

Like opium or heroin, morphine binds to the opioid receptors and is widely used to investigate the

‡ Corresponding author

* Project supported by the National Basic Research Program (973) of China (No. 2003CB515402), and the Science and Technology Council of Zhejiang Province (No. 2005C23G2010166), China

addiction to opioids (Contet *et al*., 2004; Nestler, 2004). Several animal models are established to investigate the influence of morphine, and in most of these models morphine is either given intravenously or subcutaneously by injection or infusion (Hutchings and Dow-Edwards, 1991). All these application methods are part of a chronic treatment for several days or weeks, and the impact of the opiate is usually verified by detecting the presence of morphine tolerance and morphine dependence (Hui *et al*., 1996; Itoh *et al*., 1998). Tolerance is investigated by injecting morphine acutely and checking for a reduced sensitivity regarding pain during a tail immersion or hot-plate test, while dependence is investigated by injecting the morphine antagonist naloxone and recording the expression of signs of withdrawal (e.g. wet-dog shakes, ptosis, face washing, chewing, diarrhea) (Badawy *et al*., 1982). However, although the term "morphine dependence" suggests that the observed signs reflect addiction, the naloxone injection actually only forces a physiological response to the immediate blockade of the opiate receptors and is not indicative of any drug craving behavior or uncontrolled drug intake. Hence, this approach does not reveal addiction.

In contrast to applying morphine by injection or infusion, other models feature an oral intake of the drug by adding morphine into the drinking water (Hui *et al*., 1996; Fábián *et al*., 2003). Such an approach offers the possibility to detect addiction, especially regarding the rewarding effect of the drug, much clearer—after a treatment phase and a phase of withdrawal, the animals are tested regarding their drinking preference by offering two bottles as their drinking source, with one bottle without and one with morphine (Borg and Taylor, 1994). Morphine addicted animals prefer the drug-containing solution mainly or exclusively, thus showing a clear relapse after the withdrawal phase (Dai *et al*., 1984). Such a model has already been used to investigate alcohol-addicted rats, and in the 2-bottle-choice phase, the treated animals do not only show an increase in intake, but even drink the alcohol solution after an adulteration with quinine (Wolffgramm and Heyne, 1995). Besides clearly showing the drug craving and addiction of the animal, the drinking model allows also testing certain components regarding their potential to prevent such a relapse during the 2-bottle-choice phase. Purified plant components like daidzin and daidzein from *Pueraria lobata* and ibogaine from *Tabernanthe iboga* (Rezvani *et al*., 2003) have been shown to exert an anti-dipsotropic activity on alcohol-addicted animals, and other plant components like quercetin or apigenin are currently investigated regarding their potential to reduce the impact of morphine (Gomaa *et al*., 2003; Naidu *et al*., 2003).

Several reports have described the application of morphine in the drinking water; however, they are not free of conflicting results. For example, Hui *et al*.(1996) as well as Borg and Taylor (1994) reported that morphine-treated rats preferred after a withdrawal period the morphine solution more than a morphine-free solution, but Hinson *et al*.(1986) and Jurna *et al*.(1992) could not detect this preference. In addition, while some studies indicate that morphine-drinking rats show both morphine tolerance and signs of withdrawal after injection of naloxone (Gellert and Holtzman, 1978; Hui *et al*., 1996), others could not confirm the development of tolerance (Jurna *et al*., 1992). Accordingly, this study aimed to figure out if the chronic consumption of morphine in the drinking water could lead to morphine tolerance and addiction-generating rewarding effects. For three weeks, male Wistar rats received a daily morphine dose of 35 mg/kg by offering each animal the necessary volume of 5% (w/v) sucrose water with morphine (0.1 mg/ml) for up to 6 h; animals receiving just sucrose water served as controls. After this treatment phase, all animals received only tap water for one week (withdrawal phase), and were then offered two bottles, containing either sugar water or sugar water with morphine. Despite showing a reduction in body weight and morphine tolerance in the tail immersion test immediately after the treatment phase, the morphine-treated animals expressed no preference for the opiate during the 2-bottle-choice phase and drank like the control animals mainly the sugar water. Apparently, the chronic consumption of morphine only led to morphine tolerance, but not to morphine addiction or any rewarding effect of the opiate.

MATERIALS AND METHODS

Chemical reagents

Morphine hydrochloride (Shenyang Pharma-

ceutical Ltd., Shenyang, China); sucrose (Guangdong Guanghua Chemical Factory, Guangdong, China).

Animal care

Male Wistar rats (140~165 g) were supplied by the Zhejiang Medical Science Research Institute, Hangzhou, China, and housed under conditions of controlled temperature ((22±2) °C) and lighting (reversed 12-h light/dark cycle with lights on at 06:00 p.m.). The animals were kept in cages (4 rats per cage) and had free access to food and water. After an adaptation period of 3 weeks, during which the animals with similar average body weight as well as food and water consumption, were transferred to live in cages where the rats were receiving 5% (w/v) sucrose water without (control group) or with 0.1 mg/ml morphine (morphine group) for several weeks as outlined below. All experiments were carried out at room temperature between 09:00 a.m. and 05:00 p.m.; body weight, food intake and drinking water consumption were monitored daily during the initial adaptation period and the experimental period. The experimental protocol was approved by Zhejiang University Animal Research Certificate SYXK (ZHE) 2004-0052 and is in accordance with international guidelines for care and use of laboratory animals.

Morphine administration in the drinking water

Following the acclimation period, the animals were transferred into individual cages for up to 6 h every day and received freshly prepared 5% (w/v) sucrose water either without (control group) or with a constant morphine concentration of 0.1 mg/ml (morphine group). The volume of the given fluids was restricted (50~75 ml) and calculated according to the body weight of the rats. Accordingly, a complete consumption of the volume-limited morphine solution within the given time period of 6 h resulted in a constant daily morphine intake of 35 mg/kg by the animals of the morphine group, while the control rats received a corresponding volume of sugar water during that time. The drinking was checked by measuring the volume left in each bottle every 1.5 h. After the drinking, all animals were transferred back to their group cages and socially housed for the rest of the day. The daily exposure of the rats to sugar water without or with morphine was done for 3 weeks (treatment phase); then, the animals were kept under initial conditions and received only water as their drinking source for 1 week (withdrawal phase). Afterwards, during the final 2 d of the experiment, the animals were transferred into individual cages for 3 h every day and were offered two bottles per cage, filled either with sucrose water or with sucrose water plus 0.1 mg/ml morphine (preference phase).

Evaluation of morphine tolerance

The tail immersion test was employed to detect the development of morphine tolerance. Forty minutes after an i.p. injection of either morphine (10 mg/kg body weight) or an equivalent volume of saline (control), the rats were wrapped in a soft towel and kept gently in hand. Then, the terminal 5 cm of their tails were first submerged into room temperature water (22~24 °C) to check for an aversion to water, and afterwards immersed in 52 °C water. The reaction time between immersing the tail and its removal out of the heated water was measured; a cut-off latency of 20 s was employed to avoid damaging the tail.

Statistical analysis

All values are means±*SEM*, with a *n*-value of 8. Significant differences in the experimental results were determined using Student's *t*-test.

RESULTS

Chronic morphine drinking affects body weight gain

In comparison with the control group, the daily consumption of morphine at a dosage of 35 mg/kg did cause a significant reduction of body weight during the 3 weeks of treatment (Fig.1), although both groups had free access to food. Immediately before the experiment started (day 0), the rats of the two groups had a similar body weight of (189.0±4.0) g (control) and (186.8±6.6) g (morphine). At the end of the treatment phase (day 21), the animals of the control group had an average body weight of (207.9±5.5) g and thus gained weight, while the rats of morphine group had an average body weight of (161.3±3.1) g and significantly lost weight ($P<0.01$). In the following week, during which the opiate was withdrawn, all animals were gaining weight resulting on day 28 in

an average body weight of (220.3±8.2) g and (177.8±6.3) g for control and morphine group, respectively.

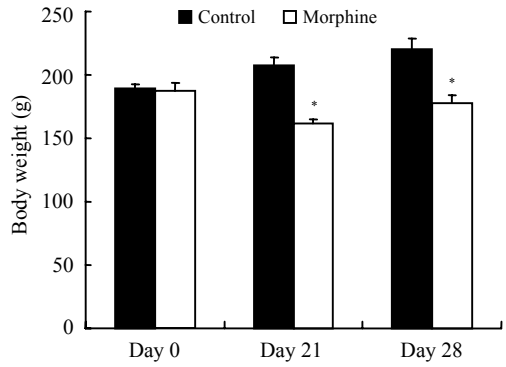

Fig.1 Body weight of Wistar rats receiving sucrose water either without (control) or with morphine (morphine)
Data (n=8 per group) are presented as mean±*SEM*; $^{*}P$<0.01 vs control

Morphine reduces the drinking of sucrose water

While Wistar rats were consuming the 5% sucrose water very quickly, the adding of morphine to the sugar water clearly affected the drinking and slowed the fluid intake down (Fig.2). Immediately before morphine was introduced (day 0), the animals of the morphine group consumed (40.3±2.9) ml of 5% sucrose-water within 3 h, thus being almost identical with the animals of the control group which drank (41.0±3.3) ml during that period. However, after adding morphine at a concentration of 0.1 mg/ml to the sugar water (day 1), the fluid intake in this group was in comparison with the control significantly reduced (P<0.01). On that day, the animals of the morphine group drank (34.5±3.1) ml within 3 h, while the control rats consumed (47.3±1.9) ml. At the end of the treatment phase (day 21), the morphine rats drank (39.8±2.7) ml sucrose water with morphine within 3 h and thus showed a similar fluid intake as in the beginning (day 0). In contrast, the control rats drank now much faster and were able to consume (69.8±2.4) ml sucrose water in the same time. Accordingly, adding sucrose at a final concentration of 5% (w/v) to the drinking water does not completely mask the bitter taste of the opiate, even at a relative low concentration of 0.1 mg/ml. However, within 6 h the animals of the morphine group were able to consume the necessary volume of the morphine solution (about 65 ml) to receive a daily morphine dose of 35 mg/kg during the entire treatment phase.

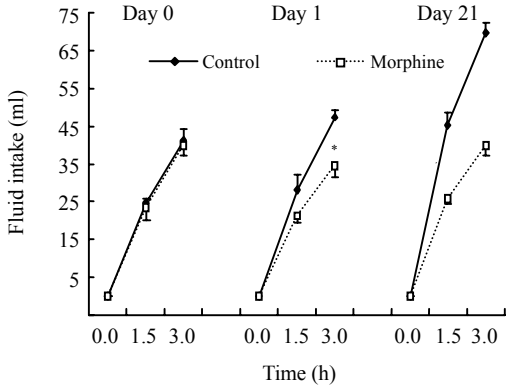

Fig.2 Fluid intake of Wistar rats receiving sucrose water either without (control) or with morphine (morphine)
Data (n=8 per group) are presented as mean±*SEM*; $^{*}P$<0.01 vs control

Chronic morphine drinking establishes tolerance, but not addiction

To check if the chronic consumption of morphine could not only lead to weight loss, but also to morphine tolerance, the animals were used for the tail immersion test. The rats of both groups responded to the noxious stimulus very quickly and removed their tail from the heated water bath within 4 s (Fig.3). After an intraperitoneal injection of morphine (10 mg/kg body weight), this reaction time was delayed. However, while the control animals removed their

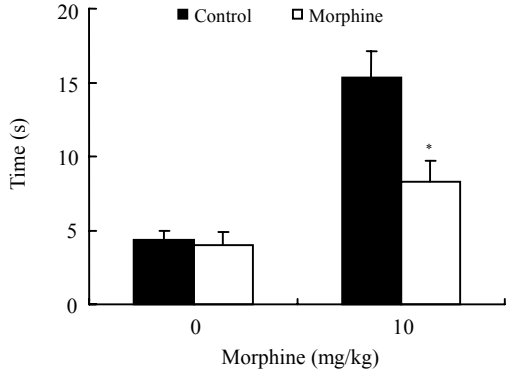

Fig.3 Tail withdrawal time after injecting either saline or morphine into Wistar rats previously receiving sucrose water without (control) or with morphine (morphine)
Data (n=8 per group) are presented as mean±*SEM*; $^{*}P$<0.01 vs control

tails after (15.3±1.8) s, the morphine treated animals showed a reaction time of (8.3±1.4) s. This significant reduction (P<0.01) of their reaction time shows that the chronically morphine-treated animals have clearly developed tolerance to the antinociceptive action of acutely administered morphine.

After withdrawing the opiate for one week (withdrawal phase), the animals of both groups were again receiving 5% sucrose water either without or with morphine (0.1 mg/ml), but this time both fluids were offered in parallel, allowing each animal to choose its drinking source for two days (2-bottle-choice phase). The morphine-treated rats drank like the control animals mainly sugar water and consumed the morphine solution only in small volumes (Fig.4). Noteworthy, the opiate-treated animals were clearly in favor of the sugar water as they consumed like the control rats at the last day (day 30) much more of this solution than the day before (day 29). As in contrast the intake of the morphine solution was even decreasing ((9.8±2.1) ml on day 29 vs (7.3±1.6) ml on day 30), it was obvious that the previous morphine-treatment could not establish any preference for the morphine solution. Accordingly, the chronic consumption of 5% sucrose water with morphine induced the development of tolerance, but not addiction-generating rewarding effects of the drug.

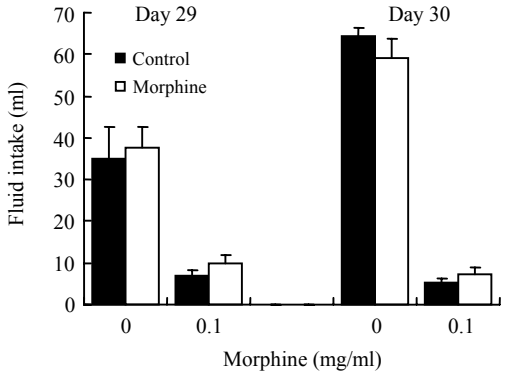

Fig.4 Drinking preference for sugar water without or with 0.1 mg/ml morphine of Wistar rats previously receiving sucrose water either without (control) or with morphine (morphine)
Data (n=8 per group) are presented as mean±*SEM*

DISCUSSION

Morphine is one commonly used substance to investigate the impact of opiates, and several ways of applying morphine in an animal model have been described, varying from injection and infusion to oral self-administration. In the last case, morphine is added into the drinking water, and although the oral intake reduces the bioavailability of morphine by 65% when compared to an application intravenously (Westerling *et al*., 1995), this method has successfully been used to investigate the expression of the μ-opioid receptors (Fábián *et al*., 2003) or the altered immune status (West *et al*., 1997) of chronically treated rats. However, those experiments were testing the animals only regarding their morphine tolerance and their behavior after naloxone injection, hence leave the question unanswered if the rats were really addicted to morphine. While some reports (Borg and Taylor, 1994; Hui *et al*., 1996) indicate that the chronic morphine drinking can indeed establish a drug-craving-like condition, other studies could not confirm this finding (Hinson *et al*., 1986; Jurna *et al*., 1992). Also the present study shows that the chronic morphine drinking does not lead to addiction due to the lack of a strong rewarding effect of the drug; however, the morphine-drinking model might give different findings according to the experimental setting of factors like the duration of treatment, the employed dose or the animal type.

Concerning the treatment period, most articles describing the use of morphine in the drinking water are consistent regarding a treatment for 3 weeks (Borg and Taylor, 1994; Fábián *et al*., 2003; Hui *et al*., 1996; West *et al*., 1997), but as the first week is normally used to increase the morphine concentration stepwise, the rats receive the final morphine concentration only for two weeks. In contrast, the Wistar rats of this study were receiving the same daily dose of morphine during the entire treatment period of 3 weeks. However, a longer treatment phase might be necessary to generate addicted animals, and a treatment period of 69 d has been reported (Silva and Heyman, 2001).

Regarding the dose, various morphine concentrations, ranging from 0.2 mg/ml to 1.2 mg/ml, have been added to the drinking water (Hinson *et al*., 1986; West *et al*., 1997). While some research group have pointed out the adding of sugar to the water is necessary to ensure the drinking (Hui *et al*., 1996), other groups showed that normal tap water with the same morphine concentration was also consumed (West *et*

al., 1997). In this study, 5% sucrose was added to the drinking water mainly to ensure a constant morphine consumption within several hours. However, the adding of morphine at a concentration of 0.1 mg/ml already affected the drinking of the rats, and a higher morphine concentration of 0.2 mg/ml in the sugar water further reduced the fluid intake (data not shown). Accordingly, it was not possible to increase the morphine concentration in the sugar water without impairing the drinking of the Wistar rats. Nevertheless, the experimental design of this study ensured that each Wistar rat received a constant daily morphine dose of 35 mg/kg for three weeks. Higher doses, ranging from 50 mg/kg to 82 mg/kg (Dai *et al*., 1984; Jurna *et al*., 1992; Silva and Heyman, 2001) have been used, but they did not always lead to addicted animals. While Hui *et al*.(1996) reported that an average dose of about 80 mg/kg was sufficient to generate morphine-preferring rats, Jurna *et al*.(1992) could not confirm such preference, even when the rats received two additional daily morphine injections to increase the dose from 80 mg/kg to 270 mg/kg.

Various rat strains like Lewis rats (West *et al*., 1997), F344 rats (Yamamoto and Mizuguchi, 1992) as well as Long Evans rats (Tiong *et al*., 1992) were all used previously in morphine-drinking experiments, but according to Dai *et al*.(1984) Sprague-Dawley rats seem to be the strain of choice as the animals showed after a treatment and withdrawal phase a preference for the morphine solution. However, it must be pointed out that the reported relapse appeared gradually over a period of 4 d and that the animals drank not exclusively from the morphine-containing bottle, raising the question if these animals were really addicted and making it worthwhile to test the Wistar rat. In general, we found that Wistar rats are more suitable for the morphine tolerance test than Sprague-Dawley rats or F344 rats (data not shown), and as the observed loss in body weight and the development of morphine tolerance indicate, Wistar rats are susceptible to the opiate and thus suitable for investigating the impact of morphine. However, we cannot exclude that another rat strain responds better to the opiate.

It is interesting to note that the morphine-drinking Wistar rats, despite having free access to food, lost weight and developed tolerance, but showed after 3 weeks of treatment no morphine preference. Hence, although weight loss and tolerance are clearly indicative of the influence of the drug, they do not necessarily reflect complete addiction and substance dependence. A commonly used method to verify morphine dependence is the injection of naloxone and a follow-up observation of the animals. This method was not used in this study to allow the animals to detoxify themselves gradually. Moreover, withdrawal signs like teeth chattering, diarrhea and writhing are not easily quantifiable (West *et al*., 1997), and, like body weight and morphine tolerance, rather indicate the impact of morphine than substance dependence. Hence, future research should also try to figure out how addiction can clearly be detected.

Future experiments investigate if the use of sugar in the drinking water might interfere with the outcome of the results or not. The large increase in the consumption of 5% sucrose-water of both animal groups on day 30 (Fig.4) could be interpreted as a relapse, indicating that the rats developed a strong preference for the sugar during the experiment. However, as the rats were offered on those days two bottles of sucrose-water (one without and one with morphine) at the same time, it is more likely that the remaining bitter taste of the morphine solution (Fig.2, day 1) prevented the intake. Also, it can be assumed that the rats had first to adapt to their new environment on day 29 and thus drank less, while on day 30, after becoming familiar with environment, drank normally. Accordingly, the increase in sucrose consumption on day 30 can be a typical behavioral response.

In summary, this study showed that chronic morphine consumption can only establish morphine tolerance, but does not necessarily lead to morphine addiction, making it necessary to re-evaluate established animal models for their potential to generate strong rewarding effects and to reflect drug addiction, especially drug craving and relapse. Future experiments have also to investigate if the use of sugar in the drinking water might interfere with the outcome of the results or not.

References

Ammon-Treiber, S., Hollt, V., 2005. Morphine-induced changes of gene expression in the brain. *Addict. Biol.*, **10**(1):81-89. [doi:10.1080/13556210412331308994]

Badawy, A.A., Evans, C.M., Evans, M., 1982. Production of tolerance and physical dependence in the rat by simple

administration of morphine in drinking water. *Br. J. Pharmacol.*, **75**:485-491.

Bailey, C.P., Connor, M., 2005. Opioids: cellular mechanisms of tolerance and physical dependence. *Curr. Opin. Pharmacol.*, **5**(1):60-68. [doi:10.1016/j.coph.2004.08.012]

Borg, P.J., Taylor, D.A., 1994. Voluntary oral morphine self-administration in rats: effects of haloperidol or ondansetron. *Pharmacol. Biochem. Behav.*, **47**(3):633-646. [doi:10.1016/0091-3057(94)90169-4]

Cami, J., Farre, M., 2003. Drug addiction. *N. Engl. J. Med.*, **349**(10):975-986. [doi:10.1056/NEJMra023160]

Contet, C., Kieffer, B.L., Befort, K., 2004. Mu opiod receptor: a gateway to drug addiction. *Curr. Opin. Neurobiol.*, **14**(3):370-378. [doi:10.1016/j.conb.2004.05.005]

Dai, S., Hui, S.C.G., Ogle, C.W., 1984. Morphine preference in rats previously morphine dependent. *Pharmacol. Res. Commun.*, **16**(5):495-511. [doi:10.1016/S0031-6989(84)80018-1]

Fábián, G., Tombor, B., Nemeth, I., Kicsi, E.G., Szikszay, M., Horvath, G., Szucs, M., 2003. Upregulation of mu opioid receptors by voluntary morphine administration in drinking water. *Acta Biol. Hung.*, **54**(2):157-166. [doi:10.1556/ABiol.54.2003.2.4]

Gellert, V.F., Holtzman, S.G., 1978. Development and maintenance of morphine tolerance and dependence in the rat by scheduled access to morphine drinking solutions. *J. Pharmacol. Exp. Ther.*, **205**:536-546.

Gomaa, A., Hashem, T., Mohamed, M., Ashry, E., 2003. *Matricaria chamomilla* extract inhibits both development of morphine dependence and expression of abstinence syndrome in rats. *J. Pharmacol. Sci.*, **92**(1):50-55. [doi:10.1254/jphs.92.50]

Hinson, R.E., Poulos, C.X., Thomas, W., Cappell, H., 1986. Pavlovian conditioning and addictive behavior: relapse to oral self-administration of morphine. *Behav. Neurosci.*, **100**(3):368-375. [doi:10.1037/0735-7044.100.3.368]

Hui, S.C., Sevilla, E.L., Ogle, C.W., 1996. Prevention by the 5-HT3 receptor antagonist, ondansetron, of morphine-dependence and tolerance in the rat. *Br. J. Pharmacol.*, **118**:1044-1050.

Hutchings, D.E., Dow-Edwards, D., 1991. Animal models of opiate, cocaine and cannabis use. *Clin. Perinatol.*, **18**:1-22.

Itoh, A., Noda, Y., Mamiya, T., Hasegawa, T., Nabeshima, T., 1998. A therapeutic strategy to prevent morphine dependence and tolerance by coadministration of cAMP-related reagents with morphine. *Methods Find. Exp. Clin. Pharmacol.*, **20**(7):619-625. [doi:10.1358/mf.1998.20.7.485728]

Jurna, I., Baldauf, J., Zenz, M., 1992. No psychological dependence after oral administration of morphine to rats. *Neurosci. Lett.*, **138**(1):77-80. [doi:10.1016/0304-3940(92)90476-N]

Koob, G.F., Sanna, P.P., Bloom, F.E., 1998. Neuroscience of addiction. *Neuron*, **21**(3):467-476. [doi:10.1016/S0896-6273(00)80557-7]

Koob, G.F., Ahmed, S.H., Boutrel, B., Chen, S.A., Kenny, P.J., Markou, A., O'Dell, L.E., Parsons, L.H., Sanna, P.P., 2004. Neurobiological mechanisms in the transition from drug use to drug dependence. *Neurosci. Behav. Rev.*, **27**:739-749.

Monteiro, M.G., 2001. A World Health Organization perspective on alcohol and illicit drug use and health. *Eur. Addict. Res.*, **7**(3):98-103. [doi:10.1159/000050727]

Naidu, P.S., Singh, A., Joshi, D., Kulkani, S.H., 2003. Possible mechanisms of action in quercetin reversal of morphine tolerance and dependence. *Addict. Biol.*, **8**(3):327-336. [doi:10.1080/13556210310001602248]

Nestler, E.J., 2004. Historical review: molecular and cellular mechanisms of opiate and cocaine addiction. *Trends Pharmacol. Sci.*, **25**(4):210-218. [doi:10.1016/j.tips.2004.02.005]

Rezvani, A.H., Overstreet, D.H., Perfumi, M., Massi, M., 2003. Plant derivatives in the treatment of alcohol dependency. *Pharm. Biochem. Behav.*, **75**(3):593-606. [doi:10.1016/S0091-3057(03)00124-2]

Silva, M.T., Heyman, G.M., 2001. Chronic morphine consumption decreases wheel running and wheel running-reinforced behavior in rats. *Pharmacol. Biochem. Behav.*, **69**(1-2):51-57. [doi:10.1016/S0091-3057(01)00498-1]

Tiong, G.K., Pierce, T.L., Olley, J.E., 1992. Sub-chronic exposure to opiates in the rat: effects on brain levels of substance P and calcitonin gene-related peptide during dependence and withdrawal. *J. Neurosci. Res.*, **32**(4):569-575. [doi:10.1002/jnr.490320412]

van den Brink, W., van Ree, J.M., 2003. Pharmacological treatments for heroin and cocaine addiction. *Eur. Neuropsychopharmacol.*, **13**(6):476-487. [doi:10.1016/j.euroneuro.2003.08.008]

West, J.P., Lysle, D.T., Dykstra, L.A., 1997. Tolerance development to morphine-induced alteration of immune status. *Drug Alcohol Depend.*, **46**(3):147-157. [doi:10.1016/S0376-8716(97)00059-8]

Westerling, D., Perrson, C., Hoglund, P., 1995. Plasma concentrations of morphine, morphine-3-glucuronide, and morphine-6-glucuronide after intravenous and oral administration to healthy volunteers: relationship to nonanalgesic actions. *Ther. Drug Monit.*, **17**:287-301.

WHO (World Health Organization), 2002. The World Health Report 2002—Reducing Risk, Promoting Healthy Life. Geneva, WHO.

Wolffgramm, J., Heyne, A., 1995. From controlled drug intake to loss of control: the irreversible development of drug addiction in the rat. *Behav. Brain Res.*, **70**(1):77-94. [doi:10.1016/0166-4328(95)00131-C]

Yamamoto, T., Mizuguchi, T., 1992. Time-dependent effects of oral morphine on autotomy following brachial nerve section in the rat. *Neurosci. Lett.*, **141**(2):166-168. [doi:10.1016/0304-3940(92)90886-C]