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Comparison of naloxone-induced platform vertical jumping in the assessment of physical dependence in morphine-dependent mice

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The use of subcutaneous implants of morphine pellets to produce morphine-dependent mice and the observance of withdrawal jumping upon administration of nalorphine was first described by Maggiolo & Huidobro (1961). Way, Loh & Shen (1969) used a modified morphine pellet to produce physical dependence in mice and quantified the degree of dependence by assessing the amount of naloxone required to precipitate jumping of the animals from a round platform. Since then many investigators have adopted the antagonist-induced jumping test as a measure of dependence in mice. However, several investigators, including us, have adopted the more convenient observance of naloxone-induced vertical jumping of mice in clear, plastic chambers (Marshall & Grahame-Smith, 1971; Saelens, Granat & Sawyer, 1971; Weissman, 1973; Takemori, Stesin & Tulunay, 1974a; Takemori, Ward & others, 1974b; Eidelberg, Erspamer & others, 1975; Smits, 1975; Cowan, 1976) instead of observing mice jump off a round platform. It has been suggested that perhaps the two types of jumping differ and results obtained by assessment of the two types of jumping may not be similar (E. L. Way, personal communication). To the present authors, this suggestion seemed unlikely since the vertical jumping test has been used satisfactorily to quantify physical dependence (Marshall & Grahame-Smith, 1971; Smits, 1975), to reliably predict the dependence capacity of narcotic drugs (Saelens & others, 1971; Takemori & others, 1974a, b) and to estimate the antagonistic potentencies of narcotic antagonists (Cowan, 1976). Also, both vertical jumping and jumping from a table by nalorphine-treated, morphine-dependent mice was described in the original report of Maggiolo & Huidobro (1961). Nevertheless, there has not been a systematic study comparing the results obtained by observance of both platform and vertical jumping in morphine-dependent mice. Therefore in an agreement with Dr E. Leong Way, we undertook this comparison.

Male Swiss-Webster mice, 25–30 g, were housed for at least one day in the experimental environment before experimentation and each mouse was used only once. The animals were made physically dependent on morphine by a procedure described earlier (Takemori & others, 1974a). In short, the animals were pretreaated with 50 mg kg^{-1} morphine sulphate three times daily for two days and 100 mg kg^{-1} three times on the third day before subcutaneous implantation of morphine pellets which contained 50 mg of morphine free base. This procedure not only decreased the mortality rate but assured us highly tolerant and dependent animals (Takemori & others, 1974a; Tulunay & Takemori, 1974). Physical dependence was quantified 72 h after the implantation of morphine pellets without the removal of the pellets.

Naloxone-induced platform jumping of morphine-

* Correspondence.

dependent mice was quantified exactly as described by Way & others (1969). Essentially, the number of mice jumping off a round platform, 35 cm in diameter and 70 cm high, were recorded after administration of various doses of naloxone. Three doses of naloxone and 7 animals for each dose were used. The median effective dose (ED50) and the 95% confidence limits of the dose of naloxone were estimated by the method of Litchfield & Wilcoxon (1949).

Quantification of naloxone-induced vertical jumping of morphine-dependent mice has also been described previously (Yano & Takemori, 1977; Huang, Yano & Takemori, 1978). Mice were placed singly into 30 × 30 cm plexiglass cylinders after administration of various doses of naloxone and the number of vertical jumps during the succeeding 15 min was recorded. A jumping response was considered positive when an animal jumped greater than 4 times during the observation period. This procedure eliminated any false positive responses. Three doses and 7 animals for each dose were used to estimate the naloxone ED50 and 95% confidence limits by the method of Litchfield & Wilcoxon (1949).

The ED50 of naloxone when assessed by platform jumping was 0·29 (0·15–0·55) mg kg^{-1} and that determined by vertical jumping was 0·19 (0·09–0·40) mg kg^{-1}. There was no significant difference between the two values. The slopes of the two dose-response curves were also similar. In addition 12 mice which were first used to observe platform jumping were then placed in cylinders to see whether or not those animals which jumped off a platform would also jump vertically. Eleven out of the 12 platform jumpers also jumped vertically. Furthermore, six animals which did not jump off the platform did not jump vertically. Also, in another group of 15 animals which were tested for vertical jumping initially, 14 out of 15 mice exhibited naloxone-induced vertical jumping but when placed on a platform, all 15 jumped off. Therefore it can be concluded that the assessment of physical dependence by the two types of jumping is nearly the same. In correspondence with Dr Way, he claimed that platform jumping is a more reliable index than vertical jumping because vertical jumping gives false positives. However, in the platform jumping test, no provisions are made to eliminate false positives. Indeed the two animals which jumped from the platform but did not jump vertically may have been false positives.

Although the jumping responses of the mice were made quantal in this study, other studies have used the number of vertical jumps by the animals (Marshall & Grahame-Smith, 1971; Saelens & others, 1971; Takemori & others, 1974a, b; Eidelberg & others, 1975; Smits, 1975; Cowan, 1976) or the number of platform jumps (Maruyama & Takemori, 1973). Our studies indicate that under the proper conditions, either platform or vertical jumping with the use of either quantal or measured responses can be considered to be equivalent when used to quantify the degree of morphine physical dependence in mice.

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