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Nonlinear isobologram and superadditive withdrawal from cocaine:cannabinoid combinations in planarians

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Abstract

Elucidation of interactions between drugs used in polydrug abuse is especially important. However, the necessary experimental conditions for precise quantitative analysis are difficult to establish. Because withdrawal effects of cocaine and the cannabinoid receptor agonist WIN 55212-2 are easily quantified in planarians, demonstration of synergistic effects ($P < 0.01$) of certain ratios of this combination was possible. This synergy, here analyzed with the latest (nonlinear) isobolographic methodology, is now quantitatively established for the first time.

Keywords

Cocaine; cannabinoid; nonlinear isobologram; planarians

Following exposure to cocaine or WIN 55212-2 ((*R*)-(+)-[2,3-Dihydro-5-methyl-3-[(4-morpholino)methyl]pyrrolo-[1,2,3-*de*]-1,4-benzoxazin-6-yl](1-naphthyl)methanone), planarians display a dose-related decrease in spontaneous locomotor velocity (*p*LMV) when placed into drug-free water, but not drug-containing water (i.e., abstinence-induced withdrawal) (Raffa and Valdez, 2001). Specific withdrawal signs are noted (Raffa and Desai, 2005) and the phenomenon is not due to changes in pH, temperature, or osmolarity. Planarians offer a particularly good model for such analysis. They have a simple nervous system (cephalic ganglia and ventral nerve cords) and they possess relevant neurotransmitter systems and respond to corresponding ligands (Carolei et al., 1975; Venturini et al., 1989). In the present work, we evaluated withdrawal from combinations of cocaine plus WIN 55212-2.

Planarians (*Dugesia dorotocephala*) were purchased from Carolina Biological Supply Co. (Burlington, NC), maintained and acclimated to temperature-controlled (21 °C) room conditions, and tested within 72 h. Each planarian was used only once. Cocaine and WIN 55212-2 were purchased from Sigma Chemical Co. (St. Louis, MO).

Planarians were placed individually into a clear plastic petri dish (14 cm diameter) containing room-temperature tap water (treated with AmQuel[®] water conditioner) located over graph paper composed of gridlines spaced 0.5 cm apart. Spontaneous locomotor velocity (*p*LMV) was quantified as the mean $\pm$ S.D. number of gridlines planarians crossed or re-crossed each

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minute over a 5-min observation period. Prior to measurement of *p*LMV, each planarian was placed into individual 0.5 ml vials for 1 h containing one of the following treatments: water, cocaine, WIN 55212-2, or fixed-ratio mixtures of cocaine and WIN 55212-2. The percent reduction in *p*LMV was taken as the effect in the construction of dose-effect curves.

The cocaine/WIN 55212-2 combination meets a requirement of joint-action analysis (Tallarida, 2000) for similar and independent action, since abstinence-induced withdrawal is observed for each tested individually. A full agonist (cocaine) in dose *B* was fitted to the equation $E = E_b B^p / (B^p + B_{50}^p)$ whereas the partial agonist (WIN 55212-2) is described by $E = E_c A^q / (A^q + A_c^q)$, where A_c denotes the dose *A* that gives half of its maximum (E_c). In these equations the exponents *p* and *q* denote constants ('Hill coefficients') that are related to the steepness of the log dose-effect curves. The isobole of additivity for the 50% effect is the set of combination dose pairs (*a*, *b*) of the partial and full agonist, respectively, and given by the equation below (Grabovsky and Tallarida, 2004):

$$b = B_{50} - \frac{B_{50}}{\left[\frac{100}{E_c} \left(1 + \frac{A_c^q}{a^q} \right) - 1 \right]^{1/p}}.$$

The dose-response curves for cocaine alone and for WIN 55212-2 alone are shown in Fig 1A. It is seen that cocaine was effective over an extended dose range and achieved the 100% effect, while WIN 55212-2 produced a lesser maximum and was effective over a narrower dose range (the figure legend gives the fitted equations). Because these agents have a variable potency ratio, the isobole of additivity is curved (Grabovsky and Tallarida, 2004; Tallarida, 2006) as shown in Fig 1B. For each of five fixed-ratio combinations complete dose-effect data were also collected from which the 50% effect level yielded the dose pairs denoted by the points shown as **A–E** in Fig 1B. Two combinations (points **A** and **B**) were found to be significantly below the additive values ($P < 0.01$) and indicate superadditivity. Point **A**, representing a fixed-ratio combination containing 23.5% WIN 55212-2 and 76.5% cocaine, has the ordinate value (cocaine component) = 0.00032 ± 0.00018 , and point **B**, containing 12.4% WIN 55212-2 and 87.6% cocaine has the ordinate 0.00166 ± 0.00067 . The *additive* ordinate corresponding to both points **A** and **B** is 0.0103 ± 0.0014 . Both experimental points are significantly below their corresponding additive values. The test for a significant difference between the combination and the additive value used the procedure described in Tallarida (2000, pp 61–62) that is based on the *t*-distribution with $P < 0.01$. These results demonstrate an important application of joint-action leading to a nonlinear isobologram.

The finding of superadditivity demonstrates that abstinence-induced withdrawal from certain combinations of cocaine and a cannabinoid receptor agonist in this model is more intense than that predicted from the individual drug potencies. This synergy, analyzed using the latest (nonlinear) isobolographic methodology, is now quantitatively established for the first time. Such findings are important for elucidating interactions in polydrug abuse.

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Fig 1.

(**A**) The dose-effect relation for WIN 55212-2 (*left*), shown against the logarithm of dose **A**,

was fitted to the equation $E = \frac{65.25A^{2.89}}{A^{2.89} + (0.000594)^{2.89}}$. The relation for cocaine in dose **B**

(*right*) was fitted to $E = \frac{100B^{0.335}}{B^{0.335} + (0.0103)^{0.335}}$. These equations permit the determination of

the additive curve described in Methods and shown in **B**. (**B**) Isobologram for the 50% effect for several combinations of cocaine and WIN 55212-2 on pLMV are shown as the five points labeled **A–E** while the smooth curve is the calculated additive isobole for this effect level. The

combinations denoted **A** and **B** are significantly below the additive curve (see text for coordinate values) indicating super-additive interactions. The other fixed-ratio combination values could not be distinguished from simple additivity.