Drug Combination Publications (RJ Tallarida *et al*)

This list of publications describes projects on ***drug combinations*** *from the laboratory of RJ Tallarida and from his collaborative work with both CSAR colleagues and external colleagues. Most projects listed were supported by both the CSAR CORE on drug combinations and data base and individual NIDA grants. The number is an identifier of the reference (that follow) of the full publication. See numbered references with summaries. References with* * *are review type articles and also appear as a separate list at the end. The* prerequisite for inclusion in this data base is the employment and description of the quantitative methodology that is required to analyze the drug interaction as synergistic or otherwise. A brief description accompanies each article that is referenced and its PubMed number is given. Articles from the *Journal of Pharmacology and Experimental Therapeutics* include the article's abstract which is provided here with the permission of that journal.

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Numbered references with brief summaries or abstract follow. Those with the symbol (*) denote review type articles containing more computational and theoretical detail.

1. *Life Sci.* 1989; 45(11):947-61. *

Statistical analysis of drug-drug and site-site interactions with isobolograms.

Tallarida RJ, Porreca F , Cowan A. #1

This paper discusses the theory of the isobologram and some new uses, viz. interactions of the same compound at different anatomical sites.

PMID 2677570

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2. *Eur J Pharmacol.* 1990;179(3):463-8

Modulation of morphine antinociception by peripheral [Leu5]enkephalin: a synergistic interaction

Porreca F, Jiang Q, Tallarida RJ: #2

This paper demonstrates synergism and discusses the interaction between mu and delta opioid agonists.

PMID 2364995

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3. *Life Sci*. 1992;50(20):1535-41.

Antinociceptive interactions of opioid delta receptor agonists with morphine in mice: supra- and sub-additivity.

Horan P, Tallarida RJ, Haaseth RC, Matsunaga TO, Hruby VJ, Porreca F. #3

This study, using mice and the warm water tail flick test, shows that these interactions can be either sub-additive or supra additive.

PMID 1315897

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4. *Pain* 49: 93-97, 1992 *

Statistical analysis of drug combinations for synergism

Ronald J. Tallarida #4

This review, dealing with drug combinations, discusses the case in which the relative potency is constant, thereby leading to a linear isobole. Calculation of the additive total dose is illustrated along with confidence limits that allow a statistical comparison with the observed total dose. Also illustrated is the case in which one of the drugs lacks efficacy.

PMID 1594286

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5. *J Pharmacol Exp Ther*. 1993 Sep;266(3):1261-7.

Isobolographic superadditivity between delta and mu opioid agonists in the rat depends on the ratio of compounds, the mu agonist and the analgesic assay used.

Adams JU, Tallarida RJ, Geller EB, Adler MW. #5

The present study was designed to test rigorously, using isobolographic analysis, whether there was a potentiative interaction between delta and mu agonists administered i.c.v. to rats. Factors such as the specific fixed ratio of compounds, the analgesic assay and the mu agonist were varied to determine the generality of the results. Male rats were implanted with i.c.v. cannulas and were tested in the cold (-3 degrees C) or hot (+50 degrees C) water tail-flick test. Full dose-effect curves were generated for the mu agonists, morphine and [N-MePhe3,D-Pro4]morphiceptin and the delta-selective agonist, DPDPE. Each agonist induced dose-related analgesia in the cold water test (ED50 values were 12, 0.79 and 75 micrograms, respectively). In the hot water test, morphine induced analgesia with an ED50 value of 4.4 micrograms, whereas DPDPE failed to produce a full effect at doses up to 200 micrograms. Full dose-effect curves were also generated for various fixed-ratio combinations of DPDPE and morphine in both analgesic assays. Fixed ratios were chosen such that the amount of DPDPE in each dose of the combinations tested was itself subanalgesic. The combination with 20% DPDPE and 80% morphine (by weight) was significantly superadditive in the cold water test as determined by isobolographic analysis, whereas a second combination of the same drugs (40% DPDPE) was not. However, in the hot water test, the 20% DPDPE combination was not superadditive and neither were two other combinations of DPDPE and morphine. No combination of DPDPE and [N-MePhe3,D-Pro4]morphiceplin differed significantly from additivity when tested in the cold water test.\

PMID 8396630

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6. *Pain* 1992 Dec;51(3):381-2; author reply 383-7.

A further comment on testing for drug synergism.

Tallarida RJ. #6

Antinociceptive and motor effects of delta/mu and kappa/mu combinations of intrathecal opioid agonists are discussed.

PMID 1491866

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7. *Life Sci.* 1996;58(2):PL 23-8. *

Testing for synergism over a range of fixed ratio drug combinations: replacing the isobologram.

Tallarida RJ , Raffa RB #7

Described here is a substitute for the isobologram in which log(total-dose) is plotted against the proportion of a component in a combination. The comparison of this method with the isobole method is discussed.

PMID 21077427

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8. *Psychopharmacology (Berl).* 1997 Oct;133(4):378-82.

Theory and statistics of detecting synergism between two active drugs: cocaine and buprenorphine.

Tallarida RJ, Kimmel HL, Holtzman SG. #8

The use of turning (due to nigrostriatal lesions) as an endpoint was employed and is unusual in quantitative studies of synergism because there is no clear maximum effect (turning). The turning data are interesting and are analyzed statistically. Synergism was detected.

PMID 9372538

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9. *Psychopharmacology (Berl).* 1997 Oct;133(4):372-7.

Synergism between buprenorphine and cocaine on the rotational behavior of the nigrally-lesioned rat.

Kimmel HL, Tallarida RJ, Holtzman SG. #9

This study provided evidence for synergism between the actions of buprenorphine and cocaine in this rat preparation.

PMID 9372537

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10. *Life Sci*. 1997;61(26):PL 417-25. *

Efficient designs for studying synergistic drug combinations.

Tallarida RJ, Stone DJ Jr, Raffa RB. #10

Here we used the composite additive dose-effect relation which allows observation of the interaction over a range of effects while reducing the size of the data sets needed.

PMID 9416783

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11. *J Pharmacol Exp Ther*. 1999 Apr;289(1):8-13.

Response surface analysis of synergism between morphine and clonidine.

Tallarida RJ, Stone DJ Jr, McCary JD, Raffa RB. #11

Abstract

Graded doses of morphine sulfate and clonidine hydrochloride were administered intrathecally to mice that were then tested for antinociception in the 55 degrees C tail immersion test. The dose-effect relations of each compound were used in calculations that permitted the construction of a three-dimensional plot of the expected additive effect (vertical scale) against the planar domain of dose pairs representing combinations administered simultaneously. This additive response surface became the reference surface for viewing the actual effects produced by three different

fixed-ratio combinations of the drugs that were used in our tests. Each combination produced effects significantly greater than indicated by the additive surface, thereby illustrating marked synergism and a method for quantifying the synergism. This quantification, measured by the value of the interaction index (alpha), was found to be dependent on the fixed-ratio combination; accordingly, the actual response surface could not be described by a single value of the index alpha. Furthermore, we found that application of the common method of isoboles gave estimates of the index that agreed well with those obtained from the more extensive surface analysis. These results confirm earlier studies, which found synergism for these drugs while also providing surface views of additivity and synergism that form the basis of isobolographic analysis.

PMID 10086981

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12. *J Pharmacol Exp Ther*. 1999 May;289(2):993-9.

Interaction between medullary and spinal delta1 and delta2 opioid receptors in the production of antinociception in the rat.

Hurley RW, Grabow TS, Tallarida RJ, Hammond DL. #12

Abstract

Previous work supports the existence of two types of delta opioid receptor (delta1 and delta2) and a role of both subtypes in the spinal cord and the ventromedial medulla (VMM) in the production of antinociception. Although it is well established that spinal and supraspinal mu opioid receptors interact in a synergistic manner to produce antinociception, little is known about the interaction of delta opioid receptors. This study used isobolographic analysis to determine how delta1 and delta2 opioid receptors in the VMM interact with their respective receptors in the spinal cord to produce antinociception. Concurrent administration of the delta1 opioid receptor agonist [D-Pen2,D-Pen5]enkephalin at spinal and supraspinal sites in a fixed-dose ratio produced antinociception in an additive manner in the tail-flick test. In contrast, concurrent administration of very low doses of the delta2 opioid receptor agonist [D-Ala2,Glu4]deltorphin at spinal and medullary sites produced antinociception in a synergistic manner. However, as the total dose of [D-Ala2,Glu4]deltorphin increased, this interaction converted to additivity. These observations suggest that different mechanisms mediate the antinociceptive effects of different doses of delta2 opioid receptor agonists. The difference in the nature of the interaction produced by delta1 and delta2 opioid receptor agonists provides additional evidence for the existence of different subtypes of the delta opioid receptor. These results also suggest that delta2 opioid receptor agonists capable of crossing the blood-brain barrier will be more potent or efficacious analgesics than delta1 opioid receptor agonists after systemic administration.

PMID 10215679

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13. *Pain* 1999 Jul;82(1):65-72.

Synergistic antiallodynic effects of spinal morphine with ketorolac and selective COX1- and COX2-inhibitors in nerve-injured rats.

Lashbrook JM, Ossipov MH, Hunter JC, Raffa RB, Tallarida RJ, Porreca F. #13

Both the warm water tail flick and nerve ligation (in rat) served as procedures for the several tests of combinations. Synergy was seen with certain combinations, but there was no synergy between morphine and ketorolac against thermal nociception in either of the tests.

PMID 10422661

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14. *Brain Res*. 1999 Jun 12;831(1-2):55-63.

Loss of antiallodynic and antinociceptive spinal/supraspinal morphine synergy in nerve-injured rats: restoration by MK-801 or dynorphin antiserum.

Bian D, Ossipov MH, Ibrahim M, Raffa RB, Tallarida RJ, Malan TP Jr, Lai J, Porreca F. #14

The restoration of supraspinal/spinal morphine antinociceptive synergy by MK-801 or dynorphin provides an interesting story.

PMID 10411983

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15. *J Pharmacol Exp Ther*. 2000 Oct;295(1):291-4.

Discovery of "self-synergistic" spinal/supraspinal antinociception produced by acetaminophen (paracetamol).

Raffa RB, Stone DJ Jr, Tallarida RJ. #15

Abstract

The mechanism of the analgesic action of one of the world's most widely used drugs-acetaminophen (paracetamol)-remains largely unknown more than 100 years after its original synthesis. Based on the present findings, this elusiveness appears to have resulted from experimental strategies that concentrated on a single target site or mechanism. Here we report on the use of analyses that we previously developed to investigate possible brain/spinal-cord site-site interaction in acetaminophen-induced antinociception. Spinal (intrathecal) administration of acetaminophen to mice produced dose-related, naloxone-insensitive antinociception with an ED(50) value of 137 (S.E. = 23) microgram = 907 (S.E. =153) nmol. In contrast, supraspinal (i.c.v.) acetaminophen administration had no effect. However, combined administration of acetaminophen in fixed ratios to brain and spinal cord produced synergistic antinociception, ED(50) = 57 (S.E. = 9) microgram, that reverted toward additivity, ED(50) = 129 (S.E. = 23) microgram, when the opioid antagonist naloxone was given spinally (3.6 microgram = 10 nmol) or s.c. (3.6 mg/kg). These findings demonstrate for the first time that acetaminophen-induced antinociception involves a "self-synergistic" interaction between spinal and supraspinal sites and, furthermore, that the self-synergy involves an endogenous opioid pathway.

PMID 10991992

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16. *Eur J Pharmacol*. 2001 Jan 26;412(2):R1-2.

Unexpected and pronounced antinociceptive synergy between spinal acetaminophen (paracetamol) and phentolamine.

Raffa RB, Stone DJ Jr, Tallarida RJ. #16

This test in mice used abdominal constriction and showed a prominent synergism between these agents.

PMID 11165231

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17. *Pain.* 2002 Jul;98(1-2):163-8. *

The interaction index: a measure of drug synergism.

Tallarida RJ. #17

This paper discusses the theory that underlies the interaction index for measuring the degree of synergism and shows that the isobolar and an alternate method can be used to calculate the index,

PMID 12098628

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18. *Br J Pharmacol.* 2002 Apr;135(7):1589-90.

Commentary on neostigmine interactions with non steroidal anti-inflammatory drugs by Miranda et al.

Tallarida RJ. #18

This commentary deals with results by Miranda et al that showed synergism between neostigmine and certain NSAID drugs (in mouse) and points out other methods of experimental design and analysis that can be used.

PMID 11934797

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19. *J Pharmacol Exp Ther.* 2002 Oct; 303(1):395-402.

N-methyl-D-aspartate antagonists and WIN 55212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1-i,j]quinolin-6-one], a cannabinoid agonist, interact to to produce synergistic hypothermia**.**

Rawls SM, Cowan A, Tallarida RJ, Geller EB, Adler MW. #19

CB(1) cannabinoid receptors mediate profound hypothermia when cannabinoid agonists are administered to rats. Glutamate, the principal excitatory neurotransmitter in the central nervous system (CNS), is thought to tonically increase body temperature by activating N-methyl-D-aspartate (NMDA) receptors. Because NMDA antagonists block cannabinoid-induced antinociception and catalepsy, intimate glutamatergic-cannabinoid interactions may exist in the CNS. The present study investigated the effect of two NMDA antagonists on the hypothermic response to WIN 55212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1-i,j]quinolin-6-one], a selective cannabinoid agonist, in rats. WIN 55212-2 (1-10 mg/kg i.m.) produced dose-dependent hypothermia that peaked 60 to 180 min postinjection. Dextromethorphan (5-75 mg/kg i.m.), a noncompetitive NMDA antagonist, or LY 235959 [(-)-6-[phosphonomethyl-1,2,3,4,4a,5,6,7,8,8a-decahydro-isoquinoline-2-carboxylate]](1-4 mg/kg i.m.), a competitive and highly selective NMDA antagonist, evoked hypothermia in a dose-sensitive manner, suggesting that endogenous glutamate exerts a hyperthermic tone on body temperature. A dose of dextromethorphan (10 mg/kg) that did not affect body temperature by itself potentiated the hypothermic response to WIN 55212-2 (1, 2.5, or 5 mg/kg). The enhancement was strongly synergistic, indicated by a 2.7-fold increase in the relative potency of WIN 55212-2. Similarly, a dose of LY 235959 (1 mg/kg) that did not

affect body temperature augmented the hypothermia associated with a single dose of WIN 55212-2 (2.5 mg/kg), thus confirming that NMDA receptors mediated the synergy. We have demonstrated previously that CB(1) receptors mediate WIN 55212-2-evoked hypothermia in rats. The present data are the first evidence that NMDA antagonists exert a potentiating effect on cannabinoid-induced hypothermia. Taken together, these data suggest that interactions between NMDA and CB(1) receptors produce synergistic hypothermia.

PMID 12235276

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20. *Am J Physiol Regul Integr Comp Physiol.* 2002 Sep;283(3):R663-8.

Interaction between muscarinic receptor subtype signal transduction pathways mediating bladder contraction.

Braverman AS, Tallarida RJ, Ruggieri MR Sr. #20

This study in rat bladder used denervated bladders to show synergistic effects of muscarinic M2 and M3 compounds.

PMID 12185001

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21. *J Pharmacol Exp Ther.* 2002 Nov;303(2):730-5.

Gabapentin and the neurokinin(1) receptor antagonist CI-1021 act synergistically in two rat models of neuropathic pain.

Field MJ, Gonzalez MI, Tallarida RJ, Singh L. #21

The present study examines the effect of combinations of gabapentin (Neurontin) and a selective neurokinin (NK)(1) receptor antagonist, 1-(1H-indol-3-ylmethyl)-1-methyl-2-oxo-2-[(1-phenylethyl)amino]ethyl]-2-benzofuranylmethyl ester (CI-1021), in two models of neuropathic pain. Dose responses to both gabapentin and CI-1021 were performed against static allodynia induced in the streptozocin and chronic constriction injury (CCI) models. Theoretical additive lines were calculated from these data. Dose responses to various fixed dose ratios of a gabapentin/CI-1021 combination were then examined in both models. In the streptozocin model, administration of gabapentin/CI-1021 combinations at fixed dose ratios of 1:1 and 60:1 resulted in an additive effect with dose response similar to the theoretical additive line. However, a synergistic interaction was seen after fixed dose ratios of 10:1, 20:1, and 40:1 with static allodynia completely blocked and the dose responses shifted approximately 8-, 30-, and 10-fold leftward, respectively, from the theoretical additive values. In the CCI model, after fixed dose ratios of 5:1 and 20:1, combinations of gabapentin and CI-1021 produced an additive response. At the fixed dose ratio of 10:1 static

allodynia was completely blocked with an approximate 10-fold leftward shift of the dose response from the theoretical additive value, indicating synergy. The combination of gabapentin with a structurally unrelated NK(1) receptor antagonist, (2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP-99,994), also produced synergy, at a fixed dose ratio of 20:1. This ratio completely blocked streptozocin-induced static allodynia and was approximately shifted leftward 5-fold from the theoretical additive value. These data suggest a synergistic interaction between gabapentin and NK(1) receptor antagonists in animal models of neuropathic pain.

PMID 12388658

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22. *J Pharmacol Exp Ther*. 2002 Nov;303(2):557-62.

Synergy between mu opioid ligands: evidence for functional interactions among mu opioid receptor subtypes.

Bolan EA, Tallarida RJ, Pasternak GW. #22

Abstract

Pharmacological differences among mu opioid drugs have been observed in in vitro and in vivo preclinical models, as well as clinically, implying that all mu opioids may not be working through the same mechanism of action. Here we demonstrate analgesic synergy between L-methadone and several mu opioid ligands. Of the compounds examined, L-methadone selectively synergizes with morphine, morphine-6beta-glucuronide, codeine, and the active metabolite of heroin, 6-acetylmorphine. Morphine synergizes only with L-methadone. In analgesic assays, D-methadone was inactive alone and did not enhance morphine analgesia when the two were given together, confirming that L-methadone was not acting through N-methyl-D-aspartate mechanisms. Both L-methadone and morphine displayed only additive effects when paired with oxymorphone, oxycodone, fentanyl, alfentanyl, or meperidine. Although it displays synergy in analgesic assays, the L-methadone/morphine combination does not exhibit synergy in the gastrointestinal transit assay. This analgesic synergy of L-methadone with selective mu opioid drugs and the differences in opioid-mediated actions suggest that these drugs may be acting via different mechanisms. These findings provide further evidence for the complexity of the pharmacology of mu opioids.

PMID 12388636

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23. *J Pharmacology Exp Ther*. 2004 Feb; 308(2):780-6. Epub 2003 Nov 10.

L-NAME (N omega-nitro-L-arginine methyl ester), a nitric-oxide synthase inhibitor, and WIN 55212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1ij]quinolin-6-one], a cannabinoid agonist, interact to evoke synergistic hypothermia.

Rawls SM, Tallarida RJ, Gray AM, Geller EB, Adler MW. #23

Cannabinoids evoke profound hypothermia in rats by activating central CB(1) receptors. Nitric oxide (NO), a prominent second messenger in central and peripheral neurons, also plays a crucial role in thermoregulation, with previous studies suggesting pyretic and antipyretic functions. Dense nitric-oxide synthase (NOS) staining and CB(1) receptor immunoreactivity have been detected in regions of the hypothalamus that regulate body temperature, suggesting that intimate NO-cannabinoid associations may exist in the central nervous system. The present study investigated the effect of N(omega)-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, on the hypothermic response to WIN 55212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenylcarbonyl)-6H-pyrrolo[3,2,1ij]quinolin-6-one], a selective cannabinoid agonist, in rats. WIN 55212-2 (1-5 mg/kg, i.m.) produced dose-dependent hypothermia that peaked 45 to 90 min post-injection. L-NAME (10-100 mg/kg, i.m.) by itself did not significantly alter body temperature. However, a nonhypothermic dose of L-NAME (50 mg/kg) potentiated the hypothermia caused by WIN 55212-2 (0.5-5 mg/kg). The augmentation was strongly synergistic, indicated by a 2.5-fold increase in the relative potency of WIN 55212-2. The inactive enantiomer of WIN 55212-2, WIN 55212-3 [S-(-)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-napthanlenyl) methanone mesylate] (5 mg/kg, i.m.), did not produce hypothermia in the absence or presence of L-NAME (50 mg/kg), confirming that cannabinoid receptors mediated the synergy. The present data are the first evidence that drug combinations of NOS blockers and cannabinoid agonists produce synergistic hypothermia. Thus, NO and cannabinoid systems may interact to induce superadditive hypothermia.

PMID 14610231

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24. *J Pharmacol Exp Ther.* 2003 Nov; 307(2):699-704. Epub 2003 Sep 9.

Antinociceptive synergy, additivity, and subadditivity with combinations of oral glucosamine plus nonopioid analgesics in mice.

Tallarida RJ, Cowan A, Raffa RB. #24

Glucosamine (2-amino-2-deoxy-d-glucose) and glucosamine-containing products have been reported to have efficacy in the treatment of various musculoskeletal disorders. Glucosamine's efficacy, including reduction of pain, is attributed to disease-modifying properties, specifically to cartilage-rebuilding associated with modulation of interleukin-1-induced activation of chondrocytes and to inhibition of proinflammatory effects of the nuclear factor-kappaB pathway. However, glucosamine has not been shown to have direct analgesic activity. We report here that commercial glucosamine (90.4% glucosamine sulfate + 9.6% excipients) administered as the sole agent (up to 500 mg/kg p.o.) was inactive in the mouse abdominal irritant test but that certain combinations of glucosamine with nonopioid analgesics at the oral doses and ratios tested resulted in a synergistic (ibuprofen and ketoprofen), additive (diclofenac, indomethacin, naproxen, and piroxicam), or subadditive (aspirin and acetaminophen) antinociceptive interaction. In the specific case of ibuprofen, the racemate (standard ibuprofen) produced dose-related antinociception with ED50 = 26.1 +/- 3.4 mg/kg. Combinations containing racemic ibuprofen and glucosamine in greater than 1:1 ratio (glucosamine/ibuprofen) were synergistic in the test (e.g., ED50 = 11.0 +/- 2.1 for the 9:1 ratio; $p < 0.01$, analysis of variance). Combinations containing glucosamine and ibuprofen (2:1 and 9:1) yielded plasma levels of ibuprofen that were no different from administration of ibuprofen alone. The possibility that combinations containing certain fixed ratios of glucosamine and certain nonsteroidal anti-inflammatory drugs (NSAIDs) might enhance pain relief in patients with pain or might achieve acceptable levels of pain relief with lower doses of NSAIDs (reduced adverse effects) is presently being pursued in clinical trials.

PMID 12966152

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25. *Eur J Pharmacol.* 2004 Feb 13;486(1):61-5.

Morphine potentiates dextromethorphan-induced vasodilation in rat superior mesenteric artery.

Inan S, Tallarida RJ. #25

An equimolar combination of morphine and dextromethorphan exhibited a marked synergism in the isolated superior mesenteric artery. The synergism was abolished by naloxone.

PMID 14751409

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26. *Expert Opin Pharmacother.* 2003 Oct;4(10):1697-708. *

Combination strategies for pain management.

Raffa RB, Clark-Vetri R, Tallarida RJ, Wertheimer AI. #26

This review discusses basic science, clinical and pharmacoeconomic aspects of pain management.

PMID 14521480

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27. *J Pharmacol Exp Ther.* 2004 Sep;310(3):981-6. *

Isobolographic analysis for combinations of a full and partial agonist: curved isoboles.

Grabovsky Y, Tallarida RJ. #27

Combinations of drugs are frequently used therapeutically to achieve an enhanced effect without using an excess quantity of either agent. If the drugs exert overtly similar action, e.g., two analgesics, the effect of the combination may be tested for additivity, i.e., an effect level that is achieved based on the individual drug potencies. But combinations of agonists will sometimes display either superadditive (synergistic) or subadditive responses. Whether the two agonists are both drugs, or a combination of a drug and an endogenous chemical, there is interest in characterizing the interaction to determine whether it departs from additivity because quantitative information of this kind, aside from its therapeutic importance, may also illuminate mechanism. A common method for this characterization uses the isobologram. This is a plot in rectangular coordinates of dose combinations (a,b) that produce the same effect level (often taken to be 50% of the maximum). In its usual form, this plot is constructed as a straight line (of additivity) connecting intercepts that represent the individually effective doses, e.g., ED50 values of each. This line is the reference for distinguishing additive from nonadditive interactions accordingly as the tested combination is on or off this line. Discussed here are the assumptions that underlie this linear plot. Specifically we show that a combination of drugs with a variable potency ratio, exemplified by a full and a partial agonist, lead to curvilinear isoboles of additivity that may erroneously be attributed to either synergism or subadditivity.

PMID 15175417

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28. *Pharmacol Biochem Behav.* 2004 May;78(1):83-91.

GABAA receptors modulate cannabinoid-evoked hypothermia.

Rawls SM, Tallarida RJ, Kon DA, Geller EB, Adler MW. #28

This study, in rats, implicates GABA(A) receptors in the hypothermic actions of cannabinoids

PMID 15159137

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29. *Life Sci.* 2005 Dec 12;78(4):329-33. Epub 2005 Oct 28.

The dynamic relationship between mu and kappa opioid receptors in body temperature regulation.

Chen X, McClatchy DB, Geller EB, Tallarida RJ, Adler MW. #29

This study, in rat, strongly suggests that mu opioid antagonists can unmask the endogenous kappa receptor-mediated hypothermia.

PMID 16257420

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30. *Eur J Pharmacol*. 2004 Oct 19;502(3):271-2.

Pronounced hypothermic synergy between systemic baclofen and NOS inhibitor.

Rawls SM, Baron DA, Gomez T, Jacobs K, Tallarida RJ. #30

This study of hypothermia in the rat showed synergism between nitric oxide synthase and baclofen.

PMID 15476754

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31. *Life Sci.* 2006 Jan 11;78(7):669-72. Epub 2005 Aug 30.

Baclofen and NOS inhibitors interact to evoke synergistic hypothermia in rats.

Rawls SM, Jacobs K, Tallarida RJ. #31

This is a more detailed study that follows the previously studied interaction of these compounds, and which explores the mechanism of the interaction.

PMID 16137704

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32. Brain Res. 2006 Oct 9;1114(1):31-5.

Subadditive withdrawal from cocaine/kappa-opioid agonist combinations in Planaria.

Raffa RB, Stagliano GW, Tallarida RJ. #32

The authors discuss their previously derived metric for quantifying withdrawal in planarians and apply it here to combinations of cocaine and a kappa opioid agonist. Measurement of withdrawal of certain fixed ratio combinations was less intense than predicted by additivity and is therefore sub-additive.

PMID 16914122

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33. *Eur J Pharmacol.* 2006 Feb 17;532(1-2):38-43. Epub 2006 Jan 30.

Effects of drug combinations on smooth muscle cell proliferation: an isobolographic analysis.

Parry TJ, Thyagarajan R, Argentieri D, Falotico R, Siekierka J, Tallarida RJ. #33

This study examines the effects of combining sirolimus with other known cell cycle-specific antiproliferative agents (cladribine, topotecan or etoposide) on cultured coronary artery VSMC proliferation and utilizes an isobolographic approach to grade the interactions. Cladribine was found to enhance the antiproliferative activity of sirolimus and was either additive or supraadditive manner, depending upon the cladribine concentration.

PMID 16448648

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34. *Eur J Pharmacol.* 2006 Jul 1;540(1-3):200-1.

Schild (apparent pA2) analysis of a kappa-opioid antagonist in Planaria.

Raffa RB, Baron DA, Tallarida RJ. #34

The interaction here is antagonism of a selective kappa agonist (U-50,488H) by a selective kappa antagonist (nor-BNI).

PMID 16737694

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35. *J Pharmacol Exp Ther.* 2006 Oct;319(1):1-7. *

An overview of drug combination analysis with isobolograms.

Tallarida RJ. #35

Drugs given in combination may produce effects that are greater than or less than the effect predicted from their individual potencies. The historical basis for predicting the effect of a combination is based on the concept of dose equivalence; i.e., an equally effective dose (a) of one will add to the dose (b) of the other in the combination situation. For drugs with a constant relative potency, this leads to linear additive isoboles (a-b curves of constant effect), whereas a varying potency ratio produces nonlinear additive isoboles. Determination of the additive isobole is a necessary procedure for assessing both synergistic and antagonistic interactions of the combination. This review discusses both variable and constant relative potency situations and provides the mathematical formulas needed to distinguish these cases.

PMID 16670349

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36. *Neuropeptides.* 2007 Apr;41(2):65-72.

Intra-VTA CART 55-102 reduces the locomotor effect of systemic cocaine in rats: an isobolographic analysis.

Jaworski JN, Kimmel HL, Mitrano DA, Tallarida RJ, Kuhar MJ. #36

The combination of CART and cocaine was found to be sub-additivie.

PMID 17289142

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37. *Pharmacol Ther.* 2007 Jan;113(1):197-209. *

Interactions between drugs and occupied receptors.

Tallarida RJ. #37

This review further analyzes isobolographic procedures and also discusses a new use of isoboles that is applicable to a single drug or chemical whose effect is mediated by 2 or more receptor subtypes.

PMID 17079019

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38. *Eur J Pharmacol.* 2006 Dec 28;553(1-3):89-98.

Agmatine and a cannabinoid agonist, WIN 55212-2, interact to produce a hypothermic synergy.

Rawls SM, Tallarida RJ, Zisk J. #38

This study, in rats, combined agmatine (50 mg/kg, i.p.) with WIN 55212-2 (1, 2.5, 5 and 10 mg/kg, i.m.) in a protocol that measured hypothermia. The enhancement was strongly synergistic, indicated by a 2.7-fold increase in the relative potency of WIN 55212-2

PMID 17109846

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39. *Pain* 2008 Feb;134(3):254-62.

Tramadol and several anticonvulsants synergize in attenuating nerve injury-induced allodynia.

Codd EE, Martinez RP, Molino L, Rogers KE, Stone DJ, Tallarida RJ. #39

This study, in rat, surgically induced allodynia and examined the combinations of each of four other anticonvulsant drugs with tramadol. Each combination showed synergism; however, the degree of synergism differed depending on the dose ratio of the constituents. The combination of tramadol and topiramate was also studied in the mouse hot-plate test and showed synergism.

PMID 17532139

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40. *Eur J Pharmacol*. 2007 Feb 5;556(1-3):89-90.

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

Raffa RB, Stagliano GW, Tallarida RJ. #40

Here it is shown that cocaine and the cannabinoid WIN 55212-2 interact synergistically in producing withdrawal effects at certain ratios. This analysis utilized the newly developed application of nonlinear isoboles.

PMID 17141755

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41. *Br J Pharmacol.* 2007 Aug;151(7):1095-102.

The beta-lactam antibiotic, ceftriaxone, attenuates morphine-evoked hyperthermia in rats.

Rawls SM, Tallarida R, Robinson W, Amin M. #41

Ceftriaxone in doses up to 200 mg/kg for 7 days did not affect the rat body temperature, but this antibiotic did attenuate the hyperthermia caused by morphine.

PMID 17592517

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42. *J Pharmacol Exp Ther*. 2008 May;325(2):567-76.

Effects of a Cannabinoid1 receptor antagonist and Serotonin2C receptor agonist alone and in combination on motivation for palatable food: a dose-addition analysis study in mice.

Ward SJ, Lefever TW, Jackson C, Tallarida RJ, Walker EA. #42

The cannabinoid and serotonin systems modulate feeding behavior in humans and laboratory animals. The present study assessed whether a cannabinoid (CB)(1) receptor antagonist and a serotonin (5-HT)(2C) receptor agonist alone and in combination attenuate motivation for the liquid nutritional drink Ensure as measured by a progressive ratio (PR) schedule of reinforcement in male C57BL/6 mice. Pretreatment (15 min i.p.) with either the CB(1) receptor antagonist N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboximide hydrochloride (SR141716) (SR; Rimonabant or Acomplia) or the 5-HT(2C) receptor agonist m-chlorophenylpiperazine (mCPP) dose-dependently decreased the maximum ratio completed under the PR schedule (break point) in mice. ED(25) values for SR and mCPP to decrease break point were determined, and the relative potency of each drug alone was quantified. Fixed dose-ratio pairs of SR/mCPP based on their relative potency were then administered. Dose-addition analysis comparing the experimentally determined potency for SR/mCPP combinations with their predicted additive potency revealed that SR/mCPP combinations in 1:1 and 2:1 ratios based on relative potency produced significant synergistic attenuation of break point for Ensure. The ED(25) values for decreasing break point were consistently lower than ED(25) values for decreasing response rate, and synergistic effects of SR/mCPP combinations on break point were seen independent of synergistic effects on response rate. These results indicate that cannabinoid CB(1) and serotonin 5-HT(2C) receptors are involved in motivated feeding behavior in mice and that these compounds can synergistically modulate motivation for palatable food with the synergy dependent upon the ratio of SR/mCPP in the combination.

PMID 18256173

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43. *Psychopharmacology (Berl).* 2008 Mar;196(4):575-82.

Self-administration of drug mixtures by monkeys: combining drugs with comparable mechanisms of action.

Woolverton WL, Wang Z, Vasterling T, Carroll FI, Tallarida R. #43

The experiment was aimed at studying self-administration of mixtures of drugs with comparable pharmacological mechanisms of action in monkeys. Two mu opioid agonists, alfentanil and remifentanil, were administered together and two dopamine (DA) uptake blockers, cocaine and RTI-117 were tested in the other group of monkeys . These combination effects were analyzed using isobolographic methods. The mixtures of doses of the two opioids or the two DA uptake blockers were tested in doses based 1:1, 1:2, and 2:1 ratios of their individual ED50s. The results for all combinations were simply additive, i.e., there was no indication of synergism.

PMID 18026937

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44. *Psychopharmacology (Berl).* 2008 Jun;198(3):387-94.

Self-administration of cocaine-remifentanil mixtures by monkeys: an isobolographic analysis.

Woolverton WL, Wang Z, Vasterling T, Tallarida R. #44

Monkeys were used to self-administer mixtures of cocaine and the mu opioid agonist remifentanil and to measure the interaction using isoboles. Several fixed ratio dose mixtures of cocaine and remifentanil were tested. The 1:1 and 1:2 coc/remi combinations approached synergism, but did not quite achieve it. However, the 2:1 combination showed synergism and thus show that interactions between stimulants and opioids can be synergistic and that this may contribute to the abuse of this combination.

PMID 18461311

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45. *J Pharmacol Exp Ther*. 2008 Jun;325(3):954-60.

The use of occupation isoboles for analysis of a response mediated by two receptors: M2 and M3 muscarinic receptor subtype-induced mouse stomach contractions.

Braverman AS, Tallarida RJ, Ruggieri MR Sr. #45

Smooth muscle contains multiple muscarinic receptor subtypes, including M2 and M3. M2 receptors outnumber M3 receptors. Based on the potency of subtype selective anticholinergics, contraction is mediated by the M3 subtype. However, results from knockout (KO) mice show that the M2 receptor mediates approximately 45% of the contractile response produced by the M3 receptor. The traditional theory of one receptor mediating a response does not allow assessment of interactions between receptors when more than one receptor participates in a response. Our study was performed using a novel analysis method based on dual receptor occupancy to determine how M2 and M3 receptor subtypes interact to mediate contraction in mouse stomach. Cumulative carbachol concentration contractile responses were determined for wild-type, M2-KO, and M3-KO stomach body smooth muscle. Using affinity constants for carbachol at M2 and M3 cholinergic receptors, the concentration values were converted to fractional receptor occupation. The resulting occupation-effect relations showed maximum effects for the M2 and M3 subtypes, respectively. These occupation-effect relations allow determination of the additive (expected) isobole based on this dual occupancy, thereby providing a curve (mathematically derived) for comparison against the experimentally derived value in wild type. The actual values determined experimentally in the wild type were not statistically significantly different from that predicted by the isobole. This confirms that the interaction between these mutually occupied receptors is additive. The new method of analysis also expands the traditional Schild theory that was based on a single receptor type to which the agonist and antagonist bind.

PMID 18339971

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46. *Eur J Pharmacol.* 2008 May 31;586(1-3):350-1.

A quantitative study to assess synergistic interactions between urotensin II and angiotensin II.

Lamarre NS, Tallarida RJ. #46

Isolated aortic rings of the rat were used and the developed tension was determined for combinations of urotensin II and angiotensin II. The interaction was synergistic. A response surface plot is given and shows the predicted additive effect for all possible combination doses and further illustrates the synergy.

PMID 18371948

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47. *J Pharmacol Exp Ther*. 2009 Apr;329(1):218-24.

Quantitation of the contractile response mediated by two receptors: M2 and M3 muscarinic receptor-mediated contractions of human gastroesophageal smooth muscle.

Braverman AS, Miller LS, Vegesna AK, Tiwana MI, Tallarida RJ, Ruggieri MR Sr. #47

Although muscarinic receptors are known to mediate tonic contraction of human gastrointestinal tract smooth muscle, the receptor subtypes that mediate the tonic contractions are not entirely clear. Whole human stomachs with attached esophagus were procured from organ transplant donors. Cholinergic contractile responses of clasp, sling, lower esophageal circular (LEC), midesophageal circular (MEC), and midesophageal longitudinal (MEL) muscle strips were determined. Sling fibers contracted greater than the other fibers. Total, M(2) and M(3) muscarinic receptor density was determined for each of these dissections by immunoprecipitation. M(2) receptor density is greatest in the sling fibers, followed by clasp, LEC, MEC, and then MEL, whereas M(3) density is greatest in LEC, followed by MEL, MEC, sling, and then clasp. The potency of subtype-selective antagonists to inhibit bethanechol-induced contraction was calculated by Schild analysis to determine which muscarinic receptor subtypes contribute to contraction. The results suggest both M(2) and M(3) receptors mediate contraction in clasp and sling fibers. Thus, this type of analysis in which multiple receptors mediate the contractile response is inappropriate, and an analysis method relating dual occupation of M(2) and M(3) receptors to contraction is presented. Using this new method of analysis, it was found that the M(2) muscarinic receptor plays a greater role in mediating contraction of clasp and sling fibers than in LEC, MEC, and MEL muscles in which the M(3) receptor predominantly mediates contraction.

PMID 19126780

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48. *Vet Anaesth Analg*. 2010 Nov;37(6):550-6.

Midazolam enhances the analgesic properties of dexmedetomidine in the rat.

Boehm CA, Carney EL, Tallarida RJ, Wilson RP. #48

This study investigated the dexmedetomidine-midazolam (D-M) interaction in the rat tail-flick test of antinociception. M showed no efficacy in the tail-flick test, and therefore a simple additive interaction of the combination should

produce effects that do not differ from the effect of D alone. However, the tail-flick latencies resulting from administration of D + M were significantly (p = 0.021) greater than those of D alone, thereby indicating a synergistic interaction between the two drugs with respect to antinociception.

PMID 21040379

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49. *Neuropharmacology.* 2009 Jun;56(8):1116-23.

Cocaine-induced hyperactivity and sensitization are dependent on GSK3.

Miller JS, Tallarida RJ, Unterwald EM. #49

This study in mice examined cocaine's effect on glycogen synthase kinase3 (GSK3) which mediates certain signaling systems. This experiment showed that cocaine activated GSK3 beta (in caudate putamen) and that this inhibition reduced the behavioral responses to cocaine.

PMID 19328817

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50. *J Clin Pharm Ther.* 2010 Jun;35(3):249-55.

Effects on the visual system might contribute to some of the cognitive deficits of cancer chemotherapy-induced 'chemo-fog'.

Raffa RB, Tallarida RJ. #50

Cognitive deficits that are associated with cancer chemotherapy (known as "chemo fog") may cause deficits in visual memory. This article discusses the possibility that these drugs have a direct action on the visual system or some interaction between this system and other brain regions.

PMID 20831527

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51. *J Pharmacol Exp Ther.* 2009 Sep;330(3):802-9.

Combinations of cocaine with other dopamine uptake inhibitors: assessment of additivity.

Tanda G, Newman AH, Ebbs AL, Tronci V, Green JL, Tallarida RJ, Katz JL. #51

Abstract

Drugs that inhibit dopamine (DA) reuptake through actions at the dopamine transporter (DAT) have been proposed as candidates for development as pharmacotherapies for cocaine abuse. Accordingly, it is important to understand the potential pharmacological interactions of cocaine with other drugs acting at the DAT. Effects of combinations of cocaine with a cocaine analog, 2beta-carbomethoxy-3beta-(4-fluorophenyl)tropane (WIN 35,428), were compared quantitatively with the combinations of cocaine with the N-butyl,4',4"-diF benztropine analog, 3-(bis(4-fluorophenyl)methoxy)-8-butyl-8-azabicyclo[3.2.1]octane (JHW 007), to determine whether their effects on DA levels in the shell of the nucleus accumbens (NAC) in mice differed. Each of the drugs alone produced dose-related elevations in NAC DA levels. In contrast to the other drugs, JHW 007 was less effective, producing maximal effects that approached 400% of control versus approximately 700% with the other drugs. In addition, the JHW 007 dose-effect curve was not as steep as those for cocaine and WIN 35,428. Combinations of cocaine with its analog, WIN 35,428, were most often greater than those predicted based on dose additivity. In contrast, combinations of cocaine with JHW 007 were most often subadditive. This outcome is consistent with recent studies suggesting that structurally divergent DA uptake inhibitors bind to different domains of the DAT, which can result in different DAT conformations. The conformational changes occurring with JHW 007 binding may result in functional outcomes that alter its abuse liability and its effects in combination with cocaine.

PMID 19483071

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52. *Drug Alcohol Depend.* 2010 Jun 1;109(1-3):126-9.

On deriving the dose-effect relation of an unknown second component: an example using buprenorphine preclinical data.

Tallarida RJ, Cowan A, Raffa RB. #52

Buprenorphine has an inverted U-shaped dose-effect curve in both rat and mouse tests of antinociception such as the tail flick test. The decrease in effect seen with the higher doses is due to an unknown second component. Application

of dose equivalence theory, shown here, allowed the derivation of the dose-effect relation of this unknown 2nd component.

PMID 20061095

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53. *Pharmacol & Ther*. 127: 165-174, 2010. *

The application of drug dose equivalence in the quantitative analysis of receptor occupation and drug combinations.

Tallarida RJ, Raffa RB. #53

This is a comprehensive review that presents the theory and computational methodology for analysis of drug combinations and dual receptor occupation.

PMID 20546783

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54. *J Pain.* 2010 Aug;11(8):701-9.

The determination and application of fixed-dose analgesic combinations for treating multimodal pain.

Raffa RB, Pergolizzi JV Jr, Tallarida RJ. #54

This article discusses the quantitative analysis and use of drug combinations for various pain conditions.

PMID 20338825

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55. *Drugs Today (Barc).* 2010 Jun;46(6):379-98.

Oxycodone combinations for pain relief.

Raffa RB, Pergolizzi JV, Segarnick DJ, Tallarida RJ. #55

This is a review article dealing with analgesic combinations that contain oxycodone.

PMID 20571607

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56. *Life Sci.* 2011 Jun 6;88(23-24):1047-54

Resveratrol in combination with other dietary polyphenols concomitantly enhances antiproliferation and UGT1A1 induction in Caco-2 cells.

Iwuchukwu OF, Tallarida RJ, Nagar S. #56

This study in Caco -2 cells examined combinations of resveratrol plus curcumin and resveratrol plus chrysin. Both combinations showed an additive interaction. Cell proliferation and UGT1A1 induction assays were used.

PMID 21466813

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57. *Pain Pract.* 2012 Feb;12(2):159-73.

Continuous multimechanistic postoperative analgesia: a rationale for transitioning from intravenous acetaminophen and opioids to oral formulations.

Pergolizzi JV Jr, Raffa RB, Tallarida R, Taylor R, Labhsetwar SA. #57

This paper reviews the safety and efficacy of oral and intravenous forms of acetaminophen with opioids during the post operative period.

PMID 21676161

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58. *J Pharmacol Exp Ther.* 2011 Apr;337(1):312-20.

Synergistic interaction between the two mechanisms of action of tapentadol in analgesia.

Schröder W, Tzschentke TM, Terlinden R, De Vry J, Jahnel U, Christoph T, Tallarida RJ. #58

The novel centrally acting analgesic tapentadol [(-)-(1R,2R)-3-(3-dimethylamino-1-ethyl-2-methyl-propyl)-phenol hydrochloride] combines two mechanisms of action, μ-opioid receptor (MOR) agonism and noradrenaline reuptake inhibition (NRI), in a single molecule. Pharmacological antagonism studies have demonstrated that both mechanisms of action contribute to the analgesic effects of tapentadol. This study was designed to investigate the nature of the interaction of the two mechanisms. Dose-response curves were generated in rats for tapentadol alone or in combination with the opioid antagonist naloxone or the α(2)-adrenoceptor antagonist yohimbine. Two different pain models were used: 1) low-intensity tail-flick and 2) spinal nerve ligation. In each model, we obtained dose-effect relations to reveal the effect of tapentadol based on MOR agonism, NRI, and unblocked tapentadol. Receptor fractional occupation was determined from tapentadol's brain concentration and its dissociation constant for each binding site. Tapentadol produced dose-dependent analgesic effects in both pain models, and its dose-effect curves were shifted to the right by both antagonists, thereby providing data to distinguish between MOR agonism and NRI. Both isobolographic analysis of occupation-effect data and a theoretically equivalent methodology determining interactions from the effect scale demonstrated very pronounced synergistic interaction between the two mechanisms of action of tapentadol. This may explain why tapentadol is only 2- to 3-fold less potent than morphine across a variety of preclinical pain models despite its 50-fold lower affinity for the MOR. This is probably the first demonstration of a synergistic interaction between the occupied receptors for a single compound with two mechanisms of action.

PMID 21262850

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59. *Eur J Pharmacol.* 2011 Nov 16;670(1):204-7.

On the quantitation of an agonist with dual but opposing components of action: application to vascular endothelial relaxation.

Lamarre NS, Parry T, Tallarida RJ. #59

The principle of dose equivalence is applied to rat aortic data for two vasoconstrictive peptides in both endothelial-denuded and intact vessels. The methodology shows how the dose-effect relations of the relaxing component and the constricting component are determined.

PMID 21910983

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60. *Eur J Pharmacol.* 2011 Sep;666(1-3):72-9.

Synergistic antihypersensitive effects of pregabalin and tapentadol in a rat model of neuropathic pain.

Christoph T, De Vry J, Schiene K, Tallarida RJ, Tzschentke TM. #60

This paper describes experiments that employed rat spinal nerve ligation to examine the pain relief among various drug combinations. Synergism was found for pregabalin and tapentadol combinations whereas a simply additive interaction was found for pregabalin with either morphine or oxycodone.

PMID 21640095

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61. Book Chapters on Drug Combinations

Tallarida RJ. Drug combinations and isoboles. In: *A Model for Drug Action and Abuse* (RB Raffa, ed). Landis Bioscience, Austin, 2008.

Tallarida RJ. Combination Analysis. In *Chemofog: Cancer Chemotherapy-Related Cognitive Deficits*, (Raffa RB and Tallarida RJ. eds.).

Landes Bioscience and SpringerScience+Business Media, New York., 2010.

Tallarida RJ. Experimental design and analysis of drug combinations. In. *Chemotherapy Induced NeuropathicPain* . (Raffa RB; Langford R; Pergolizzi,Jr JV; Porreca F; and Tallarida RJ, eds.) Chapman & Hall Boca Raton , 2012

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62. *Brain Res Bull.* 2010 May 31;82(3-4):184-7.

Inhibition of GSK3 attenuates dopamine D1 receptor agonist-induced hyperactivity in mice.

Miller JS, Tallarida RJ, Unterwald EM. #62

Glycogen synthase kinase-3 (GSK3) is associated with dopaminergic transmission. This study, in mice, examined inhibitors of GSK3 prior to a dopamine D1 receptor agonist. It asked whether GSK3 is necessary for behaviors produced by dopamine D1 receptors. The result (behavioral) suggests a role for GSK3 with the dopamine D1 receptor.

PMID 20347018

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63. *J Pharmacol Exp Ther.* 2011 Apr;337(1):312-20.

Synergistic interaction between the two mechanisms of action of tapentadol in analgesia.

Schröder W, Tzschentke TM, Terlinden R, De Vry J, Jahnel U, Christoph T, Tallarida RJ. #63

The novel centrally acting analgesic tapentadol [(-)-(1R,2R)-3-(3-dimethylamino-1-ethyl-2-methyl-propyl)-phenol hydrochloride] combines two mechanisms of action, μ-opioid receptor (MOR) agonism and noradrenaline reuptake inhibition (NRI), in a single molecule. Pharmacological antagonism studies have demonstrated that both

mechanisms of action contribute to the analgesic effects of tapentadol. This study was designed to investigate the nature of the interaction of the two mechanisms. Dose-response curves were generated in rats for tapentadol alone or in combination with the opioid antagonist naloxone or the α(2)-adrenoceptor antagonist yohimbine. Two different pain models were used: 1) low-intensity tail-flick and 2) spinal nerve ligation. In each model, we obtained dose-effect relations to reveal the effect of tapentadol based on MOR agonism, NRI, and unblocked tapentadol. Receptor fractional occupation was determined from tapentadol's brain concentration and its dissociation constant for each binding site. Tapentadol produced dose-dependent analgesic effects in both pain models, and its dose-effect curves were shifted to the right by both antagonists, thereby providing data to distinguish between MOR agonism and NRI. Both isobolographic analysis of occupation-effect data and a theoretically equivalent methodology determining interactions from the effect scale demonstrated very pronounced synergistic interaction between the two mechanisms of action of tapentadol. This may explain why tapentadol is only 2- to 3-fold less potent than morphine across a variety of preclinical pain models despite its 50-fold lower affinity for the MOR. This is probably the first demonstration of a synergistic interaction between the occupied receptors for a single compound with two mechanisms of action.

PMID 21262850

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64. *Expert Opin Pharmacother.* 2012 Jun;13(9):1261-70.

Fixed-dose combinations for emerging treatment of pain.

Raffa RB, Tallarida RJ, Taylor R Jr, Pergolizzi JV Jr. #64

This paper presents both practical and pharmacologic reasons for treating pain with drug combinations.

PMID 22420908

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65. *Genes and Cancer.* 2011 Nov;2(11):1003-8. *

Quantitative methods for assessing drug synergism.

***This publication is a review article that provides the mathematical basis for analyzing and using drug combinations*.**

Ronald J. Tallarida #65

PMID 22737266

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66. *J Pharmacol Exp Ther*. 2012 Jul;342(1):2-8 *

Revisiting the isobole and related quantitative methods for assessing drug synergism.

Tallarida RJ. #66

The isobole is well established and commonly used in the quantitative study of agonist drug combinations. This article reviews the isobole, its derivation from the concept of dose equivalence, and its usefulness in providing the predicted effect of an agonist drug combination, a topic not discussed in pharmacology textbooks. This review addresses that topic and also shows that an alternate method, called "Bliss independence," is inconsistent with the isobolar approach and also has a less clear conceptual basis. In its simplest application the isobole is the familiar linear plot in cartesian coordinates with intercepts representing the individual drug potencies. It is also shown that the isobole can be nonlinear, a fact recognized by its founder (Loewe) but neglected or rejected by virtually all other users. Whether its shape is linear or nonlinear the isobole is equally useful in detecting synergism and antagonism for drug combinations, and its theoretical basis leads to calculations of the expected effect of a drug combination. Numerous applications of isoboles in preclinical testing have shown that synergism or antagonism is not only a property of the two agonist drugs; the dose ratio is also important, a fact of potential importance to the design and testing of drug combinations in clinical trials.

PMID 22511201

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67. *Neuroscience*. 2012 Apr 19;208:79-84.

Mephedrone ("bath salt") pharmacology: insights from invertebrates.

Ramoz L, Lodi S, Bhatt P, Reitz AB, Tallarida C, Tallarida RJ, Raffa RB, Rawls SM. #67

Tests with acute mephedrone showed stereotyped movements in planarians. These were attenuated by dopamine antagonists and the abrupt withdrawal produced an abstinence –induced withdrawal response. It is concluded that that mephedrone produces behavioral effects that are associated wth psychostimulant drugs.

PMID 22300981

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68. *Pharmacology & Pharmacy* 2012, 3: 381-387

Isobolographic Method and Invertebrate (Planarian) Model for Evaluating Combinations of Waterways Pollutants

Robert B. Raffa, Deborah A. Gallo, Christopher S. Tallarida, Scott M. Rawls, Ronald J. Tallarida #68

Waterway pollutants include neostigmine bromide (from pesticides) and potassium phosphate used in fertilizers. The compounds were tested to determine the interaction for lethal effects. The result was a sub-additive interaction.

DOI: 10.4236/pp.2012.34051

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69. *Pharmacology & Pharmacy*, 2012, 3: 366-371

Disruption of Drug Effects (Dopamine, Nicotine, Pilocarpine, κ-Opioid) in Planarians by UV Light

Robert B. Raffa, Christopher S. Tallarida, Aruj Choudhry, Nadia Sanni-Adam, Sean McGonigle, Michael Baron, Zhuo L. Chen, Scott M. Rawls, Ronald J. Tallarida #69

This study in planarians presented evidence that UV light (254nm) disrupts drug receptor bonds in tests involving four different receptors. These include nicotinic cholinergic receptors, selected opioid receptors, D2 dopamine receptors and muxcarinic cholinergic receptors

DOI: 10.4236/pp.2012.33048

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70. *J Pharmacology Exp Ther.* 298: 865-872, 2001 *

Drug Synergism: Its Detection and Applications

Ronald J. Tallarida #70

Abstract

Two drugs that produce overtly similar effects will sometimes produce exaggerated or diminished effects when used concurrently. A quantitative assessment is necessary to distinguish these cases from simply additive action. This distinction is based on the classic pharmacologic definition of additivity that, briefly stated, means that each constituent contributes to the effect in accord with its own potency. Accordingly, the relative potency of the agents, not necessarily constant at all effect levels, allows a calculation using dose pairs to determine the equivalent of either agent and the effect by using the equivalent in the dose-response relation of the reference compound. The calculation is aided by a popular graph (isobologram) that provides a visual assessment of the interaction but also requires independent statistical analysis. The latter can be accomplished from calculations that use the total dose in a fixed-ratio combination along with the calculated additive total dose for the same effect. Different methods may be used, and each is applicable to experiments in which a single drug is given at two different sites. When departures from additivity are found, whether in "two-drug" or "two-site" experiments, the information is useful in designing new experiments for illuminating mechanisms. Several examples, mainly from analgesic drug studies, illustrate this application. Even when a single drug (or site) is used, its introduction places it in potential contact with a myriad of chemicals already in the system, a fact that underscores the importance of this topic in other areas of biological investigation.

PMID 11504778

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71. *Int Journal of Pure and Applied Mathematics* 71:415-425, 2011 *

Combination of drugs that produce opposite effects.

Ronald J. Tallarida, Neil S. Lamarre, Khalid Benamar #71

In this article the authors derive and illustrate the quantitative procedure for analyzing the case in which two compounds that individually produce opposite effects are given together.

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72. *Drug & Alcohol Dependence* 129(3):226-31, 2012

Cocaine synergism with alpha agonists in rat aorta: computational analysis reveals an action beyond reuptake inhibition

Neil S. Lamarre, Robert B. Raffa and Ronald J. Tallarida #72

This study, in isolated rings of rat aorta, paired cocaine with three different alpha adrenoceptor agonists. Cocaine in a low dose range produced no constriction in this classical preparation, whereas each alpha agonist displayed a clear dose-dependent measurable constriction. When cocaine (in that low dose range) was combined with each alpha agonist there was clear tension enhancement which is a vivid example of synergism.

PMID 23270987

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73. *Psychopharmacology* 231:191-8, 2014

Methylphenidate and Impulsivity: A Comparison of Effects of Methylphenidate Enantiomers on Delay Discounting

Jonathan M. Slezak, George A. Ricaurte, Ronald J. Tallarida and Jonathan L. Katz #73

Delay discounting can be used to identify treatments for ADHD and, in the dose range 1.0 to 5.6 mg/kg d-methylphendate and l-methylphenidate show synergism. These results, discussed in this paper, are important for understanding treatments of ADHD.

PMID 23975034

SUMMARY

Reviews and theory publications on Drug-Drug Combinations

(RJ Tallarida *et al*)

***An overview of drug combination analysis with isobolograms.* 35**

(*J Pharmacol Exp Ther.* 2006 Oct;319(1):1-7)

***Interactions between drugs and occupied receptors.* 37**

(*Pharmacol Ther.* 2007 Jan;113(1):197-209)

***The application of drug dose equivalence in the quantitative analysis of receptor occupation and drug combinations.* 53**

(*Pharmacol & Ther.* 127: 165-174, 2010)

***Quantitative methods for assessing drug synergism*. 65**

(*Genes Cancer.* 2011 Nov;2(11):1003-8).

Revisiting the isobole and related quantitative methods for assessing drug synergism.

66 (*J Pharmacol Exp Ther.* 2012 Jul;342(1):2-8.)

***Drug Synergism: Its Detection and Applications* 70**

(*J Pharmacology Exp Ther.* 298: 865-872, 2001)

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In: *Chemo-Fog, Cancer Chemotherapy-Related Cognitive Impairment* chapter 17 (RB Raffa,RJ Tallarida, eds), Landis Bioscience, 2010.

***Drug Combinations and Isoboles (Book Chapter)* 61A**

In: *A Model for Drug Action and Abuse* (RB Raffa, ed). Landis Bioscience, Austin, 2008

Combinations of drugs that produce opposite effects 71

(*J.Pure & Applied Math*. 71:415-425, 2011)