FLUMAZENIL-SENSITIVE DOSE-RELATED PHYSICAL DEPENDENCE IN PLANARIANS PRODUCED BY TWO BENZODIAZEPINE AND ONE NON-BENZODIAZEPINE BENZODIAZEPINE RECEPTOR AGONISTS

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Benzodiazepine (BZD) anxiolytics represent a class of medicinal agents that in addition to having therapeutic utility also present an abuse problem. Although the development of physical dependence during chronic BZD administration and the subsequent withdrawal upon cessation of their use (abstinence) in humans is generally mild, the withdrawal dysphoria can nevertheless contribute to craving and potential relapse. In this study, a sensitive metric (planarian locomotor velocity; *p*LMV) was used to quantify planarian withdrawal from two benzodiazepine class (midazolam and clorazepate) and one non-benzodiazepine class (zolpidem) BZD-receptor agonists.

Midazolam and clorazepate and zolpidem produced dose-related physical dependence, as evidenced by abstinence-induced decrease in planarian locomotor velocity (*p*LMV) when drug-exposed planarians were placed into drug-free water, but not when they were placed into drug-containing water (*i.e.*, an abstinence-induced withdrawal, because the effect was only obtained with the removal of drug and not in the continued presence of drug). We have previously shown that the decrease in *p*LMV is associated with specific withdrawal signs. In the present study, the selective benzodiazepine receptor antagonist flumazenil significantly antagonized ($P < 0.05$), by co-application, the ability of each of the agonists to produce the withdrawal.

These results suggest that (1) each of the three BZD receptor agonists produced an abstinence-induced withdrawal, (2) the withdrawal was elicited by two structural classes of BZD receptor agonist, benzodiazepine (midazolam and clorazepate) and imidazopyridine derivative (zolpidem), (3) the withdrawal from all three agonists was attenuated by the BZD receptor antagonist flumazenil, (4) physical dependence developed rapidly in this model (≤ 1h), and (5) induction of withdrawal was rapid (within minutes) following initiation of abstinence. In the absence of a cloned GABA receptor or BZD binding study, the present data are the first to suggest the existence of a mammalian-equivalent BZD receptor in *Planaria*.

Finally, our results extend the demonstration of withdrawal in planarians to the BZD receptor class of drugs and demonstrate the utility of the planarian model for detecting the signs of physical dependence.