# VALPROATE-INDUCED "WET DOG SHAKES" - A REPRODUCIBLE ANIMAL MODEL OF HUMAN COMPULSIVE BEHAVIOR : EFFECT OF ANTIDEPRESSANTS

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## Abstract

Effect of serotonin re-uptake inhibitor, clomipramine (CMI) and selective serotonin re-uptake inhibitor, fluoxetine (FLX) (10 mg/kg) administered intraperitoneally were tested on valproic acid (VPA) (300 mg/kg)-induced 'wet dog shakes' (WDSs) in rats, on day 1 and 29 (single and chronic administration of VPA). Single dose and chronic administration of VPA resulted in similar increase in the number of WDSs, demonstrating it's reproducibility. CMI and FLX pretreatment resulted in significant decreases in WDSs from day 1 to 29, where as the combination of FLX and CMI did not. Single dose CMI resulted in significant reduction in WDSs on day 1 compared to FLX and VPA conditions and chronic CMI produced significant reduction in WDSs compared to FLX, VPA and FLX plus CMI ($p < 0.0005$). Acute administration of FLX produced small but significant reduction of WDSs compared to VPA condition but chronic administration of FLX resulted in a substantial and significant decrease in WDSs.

The profound decrease in WDSs with CMI suggests a predominant role for both serotonergic and anticholinergic activity, while more significant decrease of WDS on day 29 after CMI and FLX suggests a chronic receptor down regulation with either drug. The implications of these have been discussed in relation to mechanism of action of antidepressants in valproate-induced stereotyped behaviour in rats, representing a model of compulsive phenomenon seen in humans.

**Key words :** Rat, Wet Dog Shakes (WDSs), Valproic Acid (VPA), Serotonin re-uptake inhibitors (SRIs), Selective serotonin re-uptake inhibitors (SSRIs), Clomipramine (CMI), fluoxetine (FLX)

## Introduction

Behaviorally and pharmacologically, acral lick dermatitis in dogs [1], represents an appropriate animal model of obsessive compulsive disorders(OCD), which selectively responds to clomipramine (CMI), fluoxetine (FLX) and sertraline, the clinically useful SRIs but does not respond to desimipramine, which is a nor-epinephrine (NE) uptake inhibitor. Hence, experimental paradigms that exploit behavioral as well as "serotonin hypothesis of OCD" should result in the development of novel animal models that demonstrate the same selective pharmacological response, and may hence be anticipated to serve as useful tools in detecting newer agents with potential anti-OCD activity.

The earlier evidence pointed towards the involvement of basal ganglia as the site of disturbance and hence relative excess of dopaminergic activity was considered as a common denominator in OCDs [2, 1]. However, subsequent studies indicate the involvement of increased turnover of 5-HT as revealed by studies using 5-HT agonists, precursors and its antagonists. Also, several neurotransmitters and neuromodulatory systems have been implicated in the "shake behavior" (stereotypy) exhibited by rodents [3, 4]. Shake and grooming behavior in rats and mice are mediated by orbito-frontocortical pathways and appear to be related to the function of central 5-HT mechanisms including $5\text{-HT}_1$A receptors [5, 6]. Valproic acid (VPA) a potent anticonvulsant agent, induces stereotyped movements in rats, known as "wet dog shakes" (WDSs), which may involve both GABA-ergic and 5-hydroxytryptaminergic systems, as evidenced through rapidly elevated levels of 5-HT in the rat brain [7]. In pursuance of this objective, we have earlier demonstrated time and dose dependency of VPA-induced WDSs in rats [8]. Bethanechol also induces WDS implicating cholinergic mechanisms in the induction of WDSs.

The purpose of the present investigation was mainly to assess (a) the predictive validity of VPA-induced rat WDS model and its ability to identify potential anti-OCD drugs and (b) to determine if effects of single dose and chronic administration of CMI (SRI) and FLX (SSRI), are reproducible as seen with clinical responses of these drugs in OCD. Hence, in order to pharmacologically validate the WDS rat model, we evaluated the effects of an SRI and an SSRI, both effective in the treatment of OCD.

## Material and Methods

### Animals

Wistar rats of either sex weighing 170-200 g, housed 4/cage, maintained at 28 ± 5° C, under standard conditions of 12 h light/12 h dark cycles, were used for experiments, after obtaining approval from the Animal Ethics Committee at the St. John's Medical College Hospital, Bangalore, India. Rat pellets (Hindustan Lever India Ltd.) and tap water were provided *ad libitum* except during experimental sessions. Ten rats per drug dose (5 males and 5 females) were used for vehicle control as well as test drug studies. Experiments were carried out between 8.30 h and 15.00 h.

### Drug treatment and experimental design

All drugs were dissolved in normal saline and injected intraperitoneally (*ip*). Valproic acid (VPA) from Knoll Pharmaceuticals, Mumbai, India; clomipramine (CMI) (Novartis India Ltd., Mumbai) and fluoxetine (FLX) (Micro Laboratories,

Bangalore, India) were procured as pure, fine, white powder.

Drugs VPA, CMI, FLX and CMI plus FLX were administered both as single dose on the day of the experiment and chronically for 29 days, to separate groups of rats. The test drugs, CMI and FLX, 10 mg/kg, each and CMI plus FLX, were given one hour prior to either saline or VPA. Then the parameters such as (a) time to onset of WDS (b) the number of WDS as whole body shakes with concomitant head-shakes and jerks in 10 min epochs for 60 min were recorded [8].

### Single dose experiments

The rats were divided into 4 groups with 10 rats in each group. VPA treated groups were run *in tandem* as control with the separate groups treated with test drug (n = 10/group). Following administration of VPA, 300 mg/kg, alone and after pre-treatment with test drugs, CMI and FLX, each rat was observed individually for general as well as WDS behavior over 60 min.

### Chronic experiments

The number of groups and treatment schedules including period of observation were same as the single dose experiments, except that all the drugs were administered daily for 29 days to separate groups of rats. The challenge dose of VPA, 300 mg/kg was given 60 min after the administration of the test drug, from day 1 and 29.

### Statistical analysis

The number of WDS in rats treated with VPA alone (control group) and those pretreated with test drugs CMI, FLX and CMI plus FLX, followed by VPA in various groups were independently counted. WDSs were expressed as mean ± SD (standard deviation). Repeated measure analysis of variance (ANOVA) was used to compare the 4 groups of animals on different drugs using day 1 and day 29 as the repeated measure. We used Bonferroni correction to compare individual groups using paired or Student 't' tests following significant effects on the ANOVA. A probability value of $p < 0.0125$ was used to indicate statistical significance.

## Results

### Effect of VPA on behavior

VPA produced intermittent increase in motor activity, exploratory behavior, nose digging in the bed material and restlessness during the first 5 to 10 min, followed by ataxic gait. Normal gait was regained during one-hour observation period. VPA produced predominantly characteristic whole body shakes with negligible number of head shakes and body jerks. The 'wet dog shake' (WDS) was recognized as a single vigorous rotatory movement of the head and shoulders around the long axis of the body, characteristic of a wet dog shaking off water from its body. Episodes of WDS commenced 2.35 ± 0.09 min after administration of single dose of VPA (300 mg/kg), reached a peak within 10 min, and their number gradually declined in 60 minutes .

Following chronic treatment with VPA the number of WDSs on day 1 (24.25 ± 1.35), did not differ significantly from those observed on day 29 (27.35 ± 1.9). The WDS behavior was stable and reproducible, and was hence used as a parameter for quantitative comparisons (Table - 1). Control rats treated with saline did not exhibit WDSs.

### Behavioral effects of CMI, FLX AND CMI and FLX

ANOVA revealed a significant group effect ($F=78.6$; df = 3, 46; $P < 0.0001$), time effect ($F=68.0$; df = 1,46; $p < 0.0001$) and also group versus time interaction ($F=27.3$; df = 3,46; $p < 0.0001$). Post-hoc tests for the comparison of day 1 and day 29 (Table 1) showed that CMI and FLX pretreatment resulted in highly significant decreases in WDSs from day 1 to day 29, whereas the combination of FLX and CMI did not. Single dose pretreatment with CMI resulted in significant reduction in WDSs on day 1 compared to FLX pretreatment and VPA conditions, and chronic pretreatment with CMI produced significant reduction in the number of WDSs compared to pretreatment with FLX, VPA and also FLX plus CMI conditions ($p < 0.01$). Acute administration of FLX produced small but significant reduction of WDSs compared to the VPA condition only but chronic administration of FLX resulted in a substantial and significant decrease in WDS ($p < 0.001$).

**Table 1. Wet Dog Shakes (WDS) in rats (mean ± SD and range) on day 1 and day 29 in different drug conditions**

| Condition | Day 1 | Day 29 |
|---|---|---|
| Valproic acid | 24.3 + 1.7 (20-27) | 27.4 + 5.9 (20-50) |
| Clomipramine + Valproic acid | 15.7 + 3.8 (10-21) | 0.9 + 1.3 (0-4)* |
| Fluoxetine + Valproic acid | 22.1 + 1.8 (20-25) | 7.1 + 5.4 (0-15)* |
| Fluoxetine + Clomipramine + Valproic acid | 15.3 + 4.5 (9-21) | 11.2 + 7.9 (3-27) |

* $p < 0.0005$; Significant differences between day 1 and day 29.

## Discussion

In the present study, CMI as well as CMI plus FLX significantly and substantially decreased WDSs in the acute paradigm. This did not occur when FLX was administered alone. The most likely explanation for this appears to be the anticholinergic effects of CMI. During the chronic treatment with drugs, there was a further significant decrease of the VPA-induced WDSs with CMI as well as FLX conditions. While, the most profound decrease in WDS was seen with CMI, but not with CMI and FLX combination. This can not be explained with the available data and may need further investigation into the receptor mechanisms of these drugs.

In the present study VPA-induced WDS behavior proved to be a

stable, reproducible response, occurring without decrement over 30 day period of daily VPA. Clinically VPA is a useful anti-convulsant drug with an oral $ED_{50}$ of 490 and 180 mg/kg in rats, for the Maximal Electroshock Seizure (MES) and Metrazol tests respectively. The anticonvulsant effects of VPA following a single dose appear to last longer than the WDS phenomenon, lasting only for 30 min. Potentiation of the inhibitory effects of GABA (apart from other actions) [9, 10], are believed to be implicated in the anticonvulsant actions of VPA. However, the WDS inducing behavior is claimed to be mediated by enhanced 5-HT synthesis and release, following VPA and is proposed to be the basis for stereotyped behavior [11, 12, 13]. While VPA-induced WDSs is prevented by 5-HT antagonists and depleting agents, the anticonvulsant activity remains unaffected [2]. Pharmacokinetic data have shown that both CNS and plasma concentrations of VPA are maximal within 15 min, after the administration (when the number of WDSs are at their peak) of single intraperitoneal VPA injection [18]. These findings support differential mechanisms involved in the anticonvulsant actions of VPA and the WDSs induced by VPA.

The canine acral lick model of OCD is a spontaneously occurring disease in dogs with many behavioral and compulsive features similar to human OCD. This model is considered a superior animal model of OCD, with a potential for its utility in new drug development. In a trial in dogs, selective 5-HT reuptake inhibitors, clomipramine, fluoxetine and sertraline were all effective in clinically relevant human doses, where as desimipramine a tricyclic anti-depressant with NE uptake inhibitory properties and fenfuramine, a 5-HT releasing agent, were ineffective [20]. These overall similarities in the dog and rat model of OCD, suggest that like humans, they respond specifically and selectively to 5-HT reuptake inhibitors and not to agents with different mechanisms of action.

In the present study, the acute decrease of WDSs in CMI group appears to be due to its anticholinergic effects while during the chronic condition, the effect of FLX may be due to the 5-HT receptor down-regulation. The profound and further decrease of WDSs in the chronic condition with CMI plus FLX appears to be due to the anticholinergic effects as well as 5-HT receptor down regulation. This study demonstrates that the WDS blocking effects of anti-depressant drugs CMI and FLX may indicate their effectiveness in controlling compulsive behavior in humans. In addition this study confirms and supports our earlier finding [14] that VPA-induced WDSs may serve as a simple animal model for screening drugs useful in compulsive behavior.

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