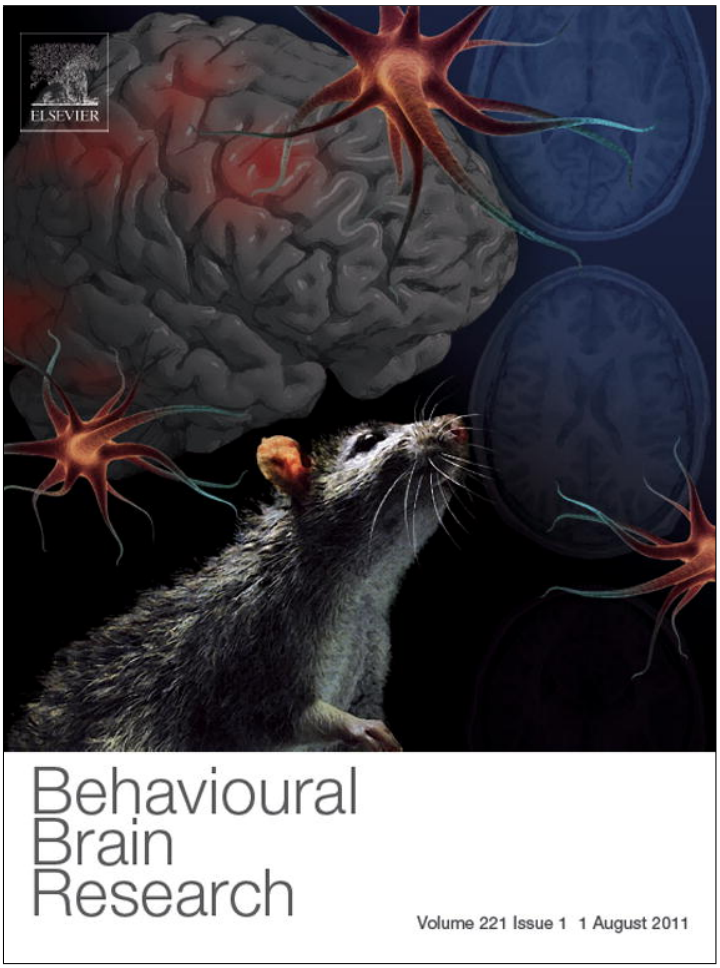

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Short communication

Triadimefon supports conditioned cue preference

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ABSTRACT

Triadimefon (TDF) is a fungicide with effects similar to cocaine, suggesting potential for abuse. Mice were trained in an apparatus with two distinctive flooring cues. In the experimental group, one of these was paired with administration of TDF and the other with vehicle; in the control group, both flooring types were paired with vehicle. The experimental group showed a significant preference shift towards the TDF-paired cue, suggesting a reinforcing effect.

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Triadimefon (TDF) is a triazole class fungicide employed for the control of fungi on agricultural and ornamental plants [28]. Triadimefon is stable in water and has the potential to leach into ground water sources [23,27] and residues have been found in commercial foods in EPA "breadbasket studies" and in the urine of agricultural workers [29]. While a number of triazole fungicides registered in the United States have been withdrawn from the market in recent years, dozens of gardening and agricultural products containing TDF as an active ingredient have been and continue to be sold world wide in more than 70 countries (see http://extoxnet.orst.edu/pips/triadime.htm; last accessed 1/27/2011).

Concerns about some of TDF's acute effects have been noted by toxicologists: it may have teratogenic [20] and carcinogenic effects [11], cause organ toxicity when fed orally [10], and be toxic to wildlife species [15]. Of particular interest, however, are the effects TDF has on behavior. Several studies have reported psychostimulant-like properties such as hyperlocomotion [24], self-mutilation at high doses [19], and increasing instrumental responding for reinforcement [2]. Similar effects have been noted with the dopaminergic drugs of abuse, cocaine, d-amphetamine, and related drugs (referred to herein as CAMP-R drugs). The comparison with CAMP-R drugs are more than surface deep, as it appears that the basis of TDF's effects on behavior is its interaction with the dopamine (DA) transporter [6,13]. By binding with the DA transporter molecule in a manner similar to cocaine, TDF increases DA concentration in the synapses, leading to acute effects similar to those of these stimulant drugs. Acute administration of high doses of TDF alter DA metabolism in the nigrostriatial and ventromedial DA pathways [28], and drug discriminations have found that TDF substitutes for (and is fully substituted by) methylphenidate, a commonly abused psychostimulant [21], suggesting that TDF may have reinforcing properties.

Because of its commonalities with CAMP-R drugs in terms of mechanisms of action and effects, it has been recognized for some time that TDF may have abuse potential [21]. In support of this notion, TDF administration produces the same kind of behavioral sensitization as cocaine and amphetamine [24]. While no instances of TDF abuse specifically have been documented to our knowledge, widespread anecdotal reports of new, legally obtainable "designer drugs" (such as mephedrone [30]) appearing in the drug-using communities in the U.S. and elsewhere suggests the importance of better characterizing the abuse potential of compounds such as TDF. In support of previous studies suggesting abuse potential, the focus of the current study was whether TDF produced some of the same kinds of subjectively rewarding effects as CAMP-R drugs in a previously unexplored task.

A behavioral paradigm commonly used for determining the extent to which subjects prefer some event is the conditioned cue preference technique, wherein a putative reinforcing event is associated with one of two or more cues. Significantly more time spent in contact with the drug-associated cue is evidence of preference. This technique and the related conditioned place preference technique, have been used to establish preference supported by food [16], water [22], social contact [5], sexual contact [1], and drugs, both illicit (e.g. cocaine [3], amphetamine [4]) and licit (e.g. nicotine [14], alcohol [7]). Should a drug have abuse potential, we should

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find that a subject should show distinct preference for stimuli associated with that drug [4].

In this study the conditioned cue preference procedure was used to determine whether pairing one tactile stimulus with TDF led to greater preference of/contact with that stimulus relative to a stimulus paired with a neutral/control event. During some days of training, confinement in the compartment with either the grid floor or the hole floor was paired with the administration of a dose of TDF, whereas on alternate days, the other flooring type was paired with a dose of vehicle. We hypothesized that TDF have the same kinds of effects as CAMP-R drugs and thus should be able to support a conditioned cue preference as evidenced by subjects spending more time in contact with the floor cue paired with TDF.

Subjects were Swiss Webster mouse females ($N = 17$, approximately 12 weeks old, weights ranging from 20–30 g), maintained in groups of 3–4 on a reversed 12:12 light schedule with unrestricted access to food and water. All animal care and protocols were in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. Public Health Service and approved by the Institutional Animal Care and Use Committee of Winona State University. Behavioral sessions were conducted in a rectangular apparatus constructed of PVC board and clear acrylic (30 cm × 15 cm × 15 cm), each of which had interchangeable floor halves consisting either of a "grid" floor (2.3-mm stainless-steel rods mounted 6.4 mm apart to form a grid) and a "hole" floor made from perforated stainless-steel with 6.4-mm round holes on 9.5-mm staggered centers. These floor types were selected due to previous studies reporting that mice preferred to spend about 50% of their time on either floor type when given a choice between the "hole" and the "grid" floors [7]. Clear acrylic was used for the front and back walls so that subjects could be videotaped during sessions.

Sessions were conducted seven days a week, under red light illumination, with one session conducted each day. On the first day of training, subjects were habituated to the apparatus by being placed inside for 5 min. Neither flooring type was employed during the habituation; instead, ordinary litter (ash shavings) covered the bottom of the apparatus. On the second day, subjects were given a pre-test in which half of the floor (left or right) was one type of flooring, whereas the other side was the other type (e.g. either grid flooring on the left and hole flooring on the right, or vice versa). Subjects were then allowed to move freely between sides for 20 min. Videotapes were scored and the amount of time spent on each floor was analyzed, yielding a percentage score for each flooring type. Subjects were considered to be on a given side of the box once they placed all four feet on that side, and remained on that side until all four feet were moved to the other side. Based on the results of the pre-test, one flooring type (the flooring type which the subject came into contact for the majority of the 20-minute pretest) was identified as "preferred", and the drug treatment was assigned to the other, "initially non-preferred" type.

Subjects then began conditioning. During each of the eight following sessions (four with each flooring type, alternating between types on succeeding days), subjects would be first be weighed, then given an i.p. injection of TDF (25 mg/kg of body weight; injection volume .005 ml/g body weight) in corn oil vehicle, or corn oil (OIL) by itself and placed immediately afterwards in the apparatus, where they remained for 10 min before being returned to home cages. On these conditioning days the apparatus was arranged with the flooring on both left and right sides being of the same type (e.g. either an "all-grid" or an "all-hole" floor.) For subjects in the experimental group, the "preferred" flooring type was consistently preceded with an injection of OIL, and the "non-preferred" type with an injection of TDF; for the control group, both floor types were preceded with an OIL injection.

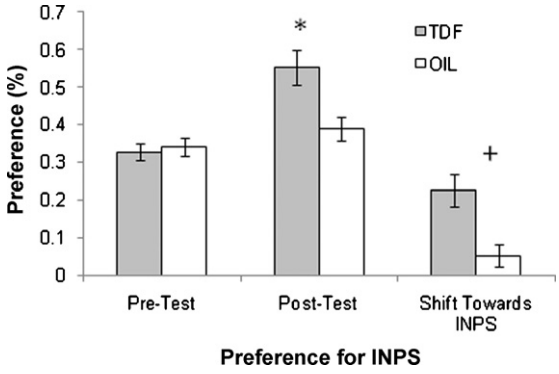

Fig. 1. Preference (in percentage) for initially no preferred side (INPS) as a function of testing condition and shift towards INPS after conditioning. (*) indicates a significant difference between initial preference and preference after conditioning, $p < .05$; (+) indicates a significant difference between TDF and OIL groups in terms of preference shift to INPS, $p < .05$.

On the eleventh day, subjects were given a post-test that was identical to the pretest, with the floor in the same "half-and-half" configuration as during the pretest, and videotaped for 20 min. This second session was scored identically to the first and the percentage of time spent on both floors established. The data of interest was how preference for the two types of flooring had changed from the pre-test to the post-test, with a significant shift in the direction of the drug-paired/initially non-preferred floor for the experimental group and no significant shift in preference for the control taken as evidence of a subjectively reinforcing effect.

Fig. 1 shows preference for the initially non-preferred/drug-paired side pre- and post-test using a number of different measures. Initial preference for the non-preferred side, recorded during the pre-test, was 33% and 34% for TDF and OIL groups, $t(15) = -42$ ns. It should be noted that subjects in both groups demonstrated an initial overall preference for the grid floor (67%) as opposed to the hole floors, contradicting previous reports that suggest subjects tend to spend 50% of their time on both floor types [7]. Preference after the conditioning phase, recorded during the post-test, was 55% and 39% for the TDF and OIL groups, $t(15) = 3$, $p < .01$. Percent shift from original (pretest) to final (posttest) preference was also compared for both groups, with that shift averaging 23% and 5% for TDF and OIL groups, $t(15) = 5.2$, $p < .01$. One sample analyses of posttest-pretest difference scores showed a significant shift in preference towards the initially non-preferred side for the TDF group, $t(6) = 5.27$, $p < .01$, but not the OIL group, $t(9) = 1.62$ ns. Overall preference for the initially non-preferred side during the post-test (as a dichotomous variable) was analyzed - for each subject, the side on which they spent more than 50% of their time during the post-test was identified. The initially non-preferred side was preferred during the post-test by 5 out of 7 subjects of the TDF group and 1 out of 10 subjects in the OIL group, $\chi^2 = 6.8$, $p < .01$ (data not shown in figure). To summarize, as hypothesized, pairing TDF with the non-preferred cue type led to a significant shift in preference towards that cue.

This study represents the first evidence that TDF exposure may be reinforcing in the same manner as cocaine, amphetamine, and other drugs of abuse. For the majority of subjects for whom drug was paired with the initially non-preferred side, there was a notable shift in preference for that side from pre- to post-test, suggesting that TDF exposure was reinforcing and that the flooring cue paired with TDF now elicited an appetitive approach response. In

fact, overall preference during the post-test was for the initially non-preferred floor for the TDF group, and all subjects - even those that ended up preferring the non-drug side after the post-test - showed a notable shift in preference towards the drug-paired floor. No comparable shift occurred for the OIL group; while some subjects showed a shift in preference, some maintained roughly the same preference, while some showed even less preference than during the pre-test.

One weakness of the current study was that a "biased" cue conditioning procedure was used. Cunningham [7] notes some inherent problems in a "biased" procedure (in which the putative reinforcing event (e.g. drug treatment, food, sexual contact, etc.) is automatically assigned to the initially non-preferred side) versus an "unbiased" procedure (in which drug treatment is randomly assigned to one cue or the other). One problem is the issue of measurement: should the putative event be assigned to the less-preferred cue, overall preference at the post-test may not be for the less-favored cue type even if the event produces a reinforcing effect (e.g. for example, if a group of subjects shows 35% preference for the less-preferred cue in the beginning, and a mildly reinforcing event is paired with that cue, in the end preference may rise to 45%, which represents a shift in the direction of the reinforcer-paired cue, but still overall preference in favor of the non-reinforced cue (e.g. 55%)). It is difficult to claim that an event is reinforcing if it does not produce a clear preference for the event-paired cue over a cue not paired with that event. This was not a problem in the current study, however, as overall preference for the drug-paired cue shifted to the initially less preferred side for the majority of subjects and for the group on average.

A more serious issue is the possibility that if a subject shows a shift in preference towards the less preferred side, that this change results not from a strictly reinforcing effect but from a drug producing an anxiolytic effect that decreases fear of the less preferred cue. The assumption made is that fear of one cue relative to the other is what drives preference and that subjects spend less time on a cue that evokes more fear in them. While the design of the current study cannot directly address this weakness, the cues employed in this study (the grid flooring and the hole flooring) were specifically chosen because past work has shown that mice tend to average roughly the same amount of time on each flooring type when given a choice between the two [7]. It seems unlikely that either floor type would be inherently more fear-producing than the other to mice.

Our findings help confirm previous suggestions that exposure to TDF is an appetitive event that elicits approach of TDF-associated cues, much as many other natural reinforcers and drugs do. Future studies of TDF might fruitfully examine other effects of exposure. It has been well-established that exposure to dopaminergic psychostimulant drugs can lead to long-lasting alterations in the brain – the so-called *incentive sensitization* phenomena [25] – as well as behavioral effects on organisms exposed *in utero* [12,18]. Animals exposed to relatively short-lasting regimens of psychostimulant drugs show alterations in reward-seeking behavior, including operant responding for food [17], increased responding in the presence of conditioned stimuli for food [31], facilitated acquisition of responding for a cocaine-paired conditioned reinforcer [8], attentional gating problems [26], and alterations in sexual behavior [9].

The fact that TDF is applied to not only lawns and ornamental plants but also comestibles raises the concern of accidental exposure in food or water. However, it should be acknowledged that the doses employed in this study represent a dosage considerably higher than what would be expected in all but the most extreme cases of environmental/accidental exposure. Of greater concern are changes that might result from deliberate use, a possibility given the reinforcing effects of TDF demonstrated herein.

In summary, this study adds weight to the conclusions of previous studies suggesting TDF may have abuse potential. However, further study is needed to characterize the behavioral effects of a range of TDF doses (as only a single dose was explored in this study), as well as further elucidating the neural mechanisms by which TDF may produce its behavioral effects. Such studies are currently underway in our laboratory.

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