

# The Normal Inhibition of Associations is Impaired by Clonidine in Tourette Syndrome

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## Abstract

**Objective:** We examined the inhibition of stimulus-stimulus associations (formally 'conditioned inhibition') in Tourette syndrome (TS). **Method:** The present study used video game style conditioned inhibition procedures suitable for children and adolescents. We tested 15 participants with a clinical diagnosis of TS in the absence of co-morbid attention deficit hyperactivity disorder and compared them with 19 typically developing age and sex matched controls (both groups aged 10–20 years). All children were tested for inhibition by summation test using two test stimuli in each of two conditioned inhibition tasks. **Results:** TS participants showed overall normal inhibition of stimulus-stimulus associations, and there was no correlation between inhibitory learning scores and symptom severity ratings. However, there was a clear reduction in conditioned inhibition in 7 TS participants medicated with clonidine. There was no significant effect of medication on excitatory learning of the stimulus-stimulus associations. **Conclusions:** We suggest that clonidine's effect on inhibitory as opposed to excitatory learning could be related to reduced noradrenergic activity. In terms of clinical implications for TS, impaired conditioned inhibition could reduce the ability of susceptible individuals to learn to control tics in the presence of associative triggers.

**Key words:** Tourette syndrome; associative learning; conditioned inhibition; clonidine

## Résumé

**Objectif:** étudier l'inhibition dans les associations stimulus-stimulus (officiellement appelées inhibitions conditionnées) chez les sujets atteints du syndrome Gilles de la Tourette (SGT). **Méthodologie:** analyse des contrôles inhibiteurs au moyen de jeux vidéos adaptés à des enfants et à des adolescents. Quinze sujets ayant reçu un diagnostic de SGT sans comorbidité de TDAH ont été comparés à 19 témoins ne présentant aucun retard de développement; les sujets, qui étaient appariés selon l'âge et le sexe, avaient entre 10 et 20 ans. Un test d'inhibition sommaire a été administré à tous les enfants; chaque tâche axée sur l'inhibition conditionnée faisait appel à deux stimuli. **Résultats:** l'inhibition globale des associations stimulus-stimulus était normale chez les sujets Tourette; il n'y avait aucune corrélation entre les notes d'apprentissage des contrôles inhibiteurs et les notes de sévérité des symptômes. Toutefois, on a constaté une nette baisse des contrôles inhibiteurs chez sept sujets Tourette qui prenaient de la clonidine. La médication n'avait aucun effet significatif sur l'apprentissage des contrôles excitateurs dans les associations stimulus-stimulus. **Conclusion:** l'effet opposé de la clonidine entre l'apprentissage des contrôles inhibiteurs et l'apprentissage des contrôles excitateurs pourrait, selon nous, être lié à une réduction de l'activité noradrénergique. Au niveau clinique, le manque d'inhibition conditionnée pourrait limiter la capacité des sujets à apprendre par association à maîtriser leurs tics en présence d'événements déclencheurs.

**Mots clés:** syndrome de Tourette, apprentissage par association, inhibition conditionnée, clonidine

**Abbreviations:** CI = conditioned inhibitor; CS = conditioned stimulus; CSt = transfer stimulus; Sg = generalized stimulus; TS = Tourette syndrome; UCS = unconditioned stimulus; YGTSS = Yale Global Tic Severity Scale.

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## Introduction

Tourette syndrome (TS) is a developmental disorder characterized by involuntary, repetitive, stereotypic tics, both motor and vocal (Albin and Mink, 2006, Chowdhury, 2008, Jankovic, 2001, Leckman, 2003, Robertson, 2000, 2006, Sheppard et al., 1999, Spencer et al., 1998, Swerdlow, 2001, The Tourette Syndrome Classification Study Group, 1993). Based on the presenting symptoms, inhibitory deficits are thought to be fundamental to TS (Brand et al., 2002, Comings and Comings, 1987, Georgiou et al., 1995, Gilbert et al., 2004, Sheppard et al., 1999, Swerdlow et al., 1996). Accordingly, inhibitory processes have been a focus of experimental studies of TS. However, the majority of investigations into cognitive and behavioral processes in TS have failed to demonstrate any significant differences compared to matched controls in participants without co-morbid attention deficit hyperactivity disorder. For example, participants with TS have been reported to perform as normal on Go/NoGo measures of response inhibition (Roessner et al., 2008, Serrien et al., 2005). Similarly, unless the inhibitory demands of the experimental task are increased, TS participants show no significant performance deficits in the color-word Stroop task or the flanker task (Channon et al., 2003, 2006, 2009).

Thus the experimental evidence suggests that TS involves more than simple deficits in response inhibition. Moreover, when performance on procedural learning tasks was systematically compared with that on a task requiring associative learning (stimulus-stimulus as well as stimulus-response) in TS, the underlying learning systems were found to be dissociable (Marsh et al., 2005). Stimulus-stimulus associations provide a mechanism through which environmental events can act as symptom triggers and have been suggested to explain variability in the frequency of symptoms in a range of disorders (Ferguson and Cassaday, 1999, Lishman, 1987, Siegel, 1977, Stewart et al., 1984, Watson, 1924). In TS, tics vary markedly in frequency and severity over the course of a day (Jankovic, 2001, Leckman, 2003, The Tourette Syndrome Classification Study Group, 1993). This variation is predictable where tics are triggered by certain life situations, moreover motor and phonetic tics are often preceded by premonitory sensations (such as ‘burning’ of the eye before a eye blink tic, sore throat preceding grunting), alleviated by the performance of the tic (Conelea and Woods, 2008; Jankovic, 2001, Leckman, 2003, Leckman et al., 1993, Prado et al., 2008, The Tourette Syndrome Classification Study Group, 1993). Thus environmental events and premonitory sensations provide a source of stimuli that could become associated with tic-generated stimuli through stimulus-stimulus associations. Such antecedent stimuli have recently been targeted in behavioral treatments for TS (Conelea and Woods, 2008, Verdellen et al., 2008, Woods et al., 2009).

An earlier study of ‘latent inhibition’, in which stimulus pre-exposure should reduce stimulus-stimulus associative learning, found this effect to be normal in TS participants (Swerdlow et al., 1996). However, although so-called latent inhibition procedures retard later learning they do not render the pre-exposed stimulus truly inhibitory (Baker and Mackintosh, 1977). True inhibition is rather demonstrated by establishing a stimulus selectively to predict the occasions on which an otherwise expected outcome will not occur (Pavlov, 1927, Rescorla, 1969). This inhibitory learning is a well-established phenomenon in the animal literature and is known to be modulated by the catecholamines (Harmer and Phillips, 1999; Tobler et al., 2003).

To date, no research has explicitly examined the inhibition of stimulus-stimulus associations (formally ‘conditioned inhibition’) in disorders such as TS. In conditioned inhibition procedures, a conditioned stimulus (CS) is presented immediately prior to an unconditioned stimulus (UCS), except on those occasions when it is preceded by the conditioned inhibitor (CI). Thus the CI comes to inhibit the CS-UCS association. We have developed video game style conditioning procedures that demonstrate reliable conditioned inhibition and are suitable for younger participants (Migo et al., 2006). Developmentally, tics typically onset between the ages of 3 and 8 years, peak early in the teens, and reduce by the age of 19 or 20 years (Chang et al., 2004, Chowdhury, 2008, Dooley et al., 1999, Leckman, 2003, Leckman et al., 1998). In the present study, we therefore tested conditioned inhibition in children and adolescents with a clinical diagnosis of TS (in the absence of co-morbid attention deficit hyperactivity disorder) and typically developing age and sex matched controls. As discussed above, the experimental evidence for inhibitory deficits in TS is inconsistent. Nonetheless, based on the evidence that inhibitory deficits are fundamental to TS, the *a priori* hypothesis under test in the present study was that participants with TS would show impaired inhibition of stimulus-stimulus associations.

Medication is indicated where tics cause significant interference with normal daily function, and traditionally, in line with the established role of the basal ganglia (Cheon et al., 2004, Giedd et al., 2001, Hyde et al., 1995, Mink, 2001, Minzer et al., 2004, Peterson et al., 1998, Sheppard et al., 1999, Stern et al., 2000), dopamine antagonists have been used (Gilbert, 2006; Srouf et al., 2008). Moreover, medication (with neuroleptics) has previously been reported to improve the learning of stimulus-response associations in experimental studies of TS (Marsh et al., 2004). However, because of concern over side effects with dopamine antagonists, a variety of other medications have been used in TS; for example, clonidine which acts as a noradrenergic alpha-2 agonist (Srouf et al., 2008). This action is of a priori interest given the

**Table 1 Demographics, medication and symptom scores for the TS participants.**

Subjects	Age (months)	Sex	Medication		YGTSS			
			Type	Dosage (mcg)	Motor	Phonic	Impairment	Total score
TS-1	137	Male	Clonazepam	500	14	13	25	52
TS-2	209	Male	(Aripiprazole)	2.5*	n/a	n/a	n/a	66
TS-3	176	Female	Clonidine	25-50	0	0	0	0
TS-4	151	Male	(Never)	n/a	5	0	0	5
TS-5	151	Male	(Clonidine)	50*	5	0	0	5
TS-6	163	Male	Clonidine	100	11	0	20	31
TS-7	161	Female	Clonidine	75-100	11	10	15	36
TS-8	193	Male	Clonidine	50	9	9	0	18
TS-9	197	Male	(Clonidine)	200*	7	2	20	29
TS-10	155	Male	Clonidine	50	18	11	10	39
TS-11	127	Male	(Never)	n/a	12	9	10	31
TS-12	190	Male	Clonidine	50	15	14	30	59
TS-13	247	Male	Aripiprazole	n/a	13	19	15	47
TS-14	121	Male	Clonidine	50	5	0	0	5
TS-15	136	Female	(Clonidine)	n/a*	14	14	10	38

TS = Tourette Syndrome participant code; mcg = micrograms medication dosage per day; YGTSS = Yale Global Tic Severity Scale; n/a = data not available or not applicable. \*Previous medication dosages; previous medication types indicated in brackets (i.e. participant was not under any medication at test); never = never medicated for TS.

body of evidence to suggest a role for noradrenalin as a key modulator of behavioural inhibition and anxiety (Gray, 1982; Nigg, 2000). In the present study, the effects on conditioned inhibition of medication with clonidine for TS were also examined.

## Methods and Materials

### Participants

15 TS participants (12 males, 3 females, mean age = 13 years 11 months, range = 10–20 years; Table 1) were recruited for the current study (The Child and Adolescent Clinic, Psychiatry Department, Queen's Medical Centre, Nottingham). All met DSM IV criteria for TS in the absence of comorbid ADHD. TS participants were also assessed using the Yale Global Tic Severity Scale (YGTSS) within 2 months of testing in the present study (same day scores were available for 11/15 participants). Diagnoses were made by a consultant psychiatrist (CP) and qualified members of his team. Additional assessments were conducted by a research nurse. The available IQ scores (for n=12 TS participants) had been measured using the Wechsler Abbreviated Scale of Intelligence (vocabulary and matrix subscales).

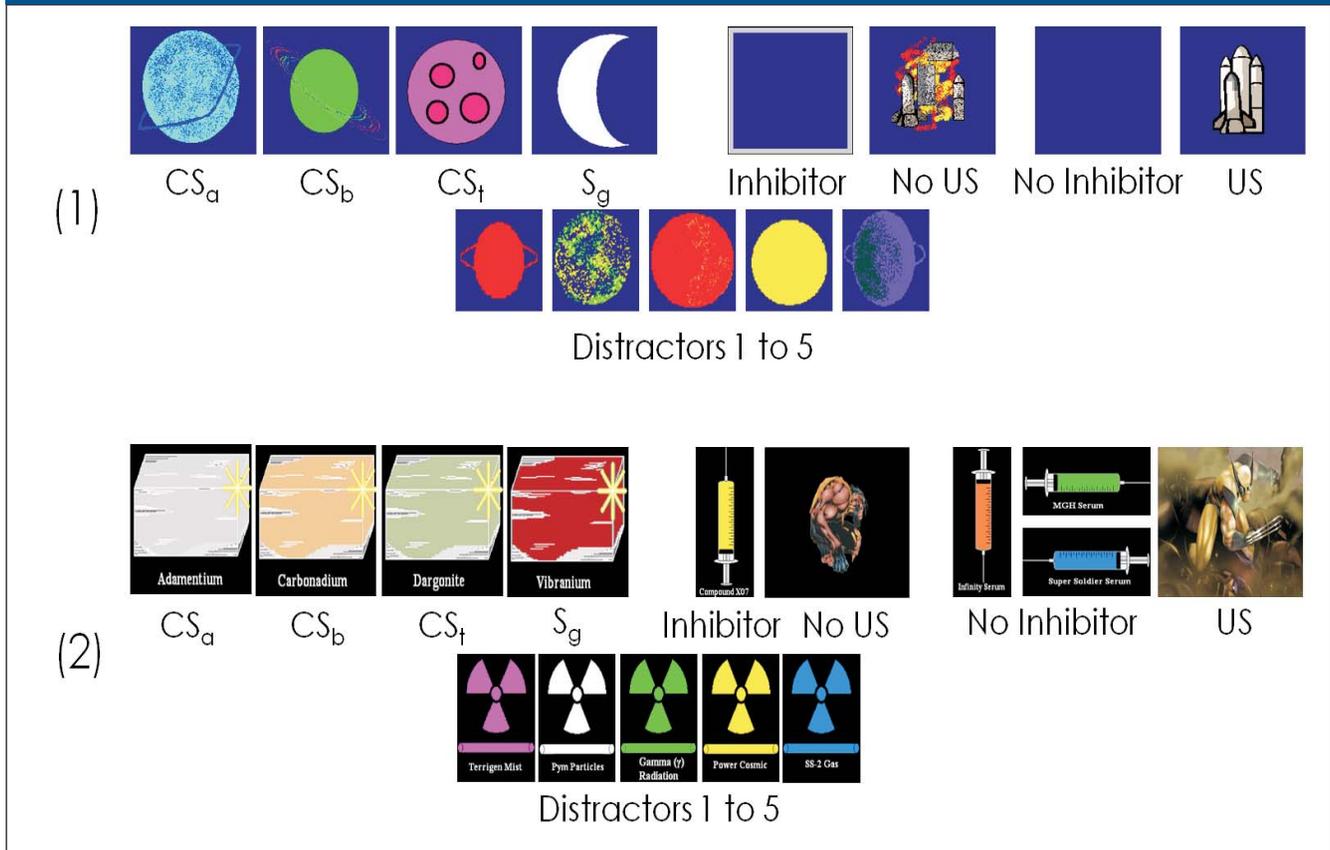
With respect to medication at the time of testing, seven TS participants were on clonidine (25–100 mcg), one was on

clonazepam (500 mcg), one was on the atypical antipsychotic aripiprazole, three had previously been on clonidine but had been medication free for a minimum of 4 months prior to the study (doses 50-200 mcg), one was drug free that day (previously treated with aripiprazole), and two had never received any medication for their TS symptoms (Table 1).

Of 35 potential controls tested, 19 were selected to provide the best match for age (within 6 months as measured on the day of testing) and sex with the TS participants. This yielded a control sample of 15 males and 4 females (mean age = 13 years 5 months, range = 10–20 years). None of the controls had family members with TS. In addition, they were screened using the Strengths and Difficulties Questionnaire (copyright Robert Goodman, 2005; Goodman, 1997). Although some difficulties were rated 'somewhat true' in the matched control participants there were no abnormal scores indicative of any undiagnosed illness; similarly, the matched controls were not on any psychotropic medication for TS, or for any other condition.

This study was approved by NHS Research Ethics (Derbyshire REC, reference 08/H0401/34, approved April 2008). All participants received an inconvenience allowance of £5-£10 to cover their travel expenses. Written consent was obtained from participants over the age of 16, both parents

Figure 1. The stimuli presented during (1) Mission-to-Mars and (2) Weapon-X task.



CS<sub>a</sub> and CS<sub>b</sub> = trained conditioned stimuli, also presented together with the inhibitor during training; CS<sub>f</sub> = transfer conditioned stimulus, not trained with the inhibitor; S<sub>g</sub> = generalized stimulus, not introduced at training, new at test; US = unconditioned stimulus.

and children signed the consent forms where participants were under 16 years.

### Materials

The task programs were produced in E-studio and utilized E-prime (Psychology Software Tools Inc., Pittsburgh, USA) to present the stimuli to the participants (Figure 1). The programs were run on personal computers with 17" monitors or on a portable 15" laptop computer when travel to the participant was required for testing.

### Procedure

#### Conditioned inhibition task 1: Mission-to-Mars

The task scenario was based on a hypothetical mission to Mars. Participants were informed that they were to play the role of a commander of a fleet of starships travelling on an exploration mission to Mars; on the course of their mission, spaceships in the fleet are prone to explode. During the training phase there were no explicit learning instructions, participants were simply asked to carefully count the number of

surviving rockets. There were 9 cycles of the 5 stimulus sequences shown in Table 2, presented in a random order. On inhibited trials, a 1-second gray frame surrounding a blue screen was presented. This was the CI. On excitatory trials, there was a 1-second presentation of an empty blue screen (at the equivalent point in the stimulus sequence). Next the CS (a large planet) was followed by 3 distractors (smaller planets) appearing and disappearing on the same screen, for a combined total of 4 seconds, then the UCS (i.e. rocket presentation) on excitatory trials or absence of the UCS (i.e. exploded rocket presentation) on inhibited trials. Participants were required to click the mouse to continue.

The subsequent test phase consisted of 20 further trials. There were 5 cycles of the 4 stimulus sequences shown in Table 2, presented in a random order. The procedure for test trials was identical to that used in training, except that prior to the presentation of the US or its absence, participants were presented with an on-screen rating, scaled 1-9. At this point, they were required to estimate the likelihood of the spaceship surviving versus exploding, with a rating of 9 to represent the highest

**Table 2 The stimulus combinations presented during the training and testing phase of the two tasks**

Training phase	Testing phase
CS <sub>a</sub> + [CI, CS <sub>a</sub> ]-	CS <sub>t</sub> + [CI, CS <sub>t</sub> ]-
CS <sub>b</sub> + [CI, CS <sub>b</sub> ]-	S <sub>g</sub> + [CI, S <sub>d</sub> ]-
CS <sub>t</sub> + [CI, CS <sub>t</sub> ]-	

Example stimuli are shown in Figure 1. CS<sub>a</sub> and CS<sub>b</sub> = trained conditioned stimuli, also presented together with the inhibitor during training; CI = conditioned inhibitor; CS<sub>t</sub> = transfer conditioned stimulus, not trained with the inhibitor; S<sub>g</sub> = generalized stimulus, not introduced at training, new at test; '+' indicates the presentation of the US (i.e. an intact rocket for the Mission-to-Mars and a picture of Wolverine for the Weapon-X task); '-' indicates the absence of the US (represented as an exploded rocket for the Mission-to-Mars and a picture of feral Logan for the Weapon-X task).

likelihood, and a rating of 1 to represent the lowest likelihood of survival. An intermediate rating of 5 represented uncertainty. Ratings were made by clicking in the appropriate on screen box using the mouse.

This was a summation test of conditioned inhibition. Participants' ratings provided a measure of the inhibitory properties of the CI using two kinds of test stimuli: (1) generalized (S<sub>g</sub> from the same category but not introduced at training); and (2) transfer (CS<sub>t</sub> familiar from training but not previously presented with the CI).

### **Conditioned inhibition task 2: Weapon-X**

This task was presented with a scenario based on the Weapon-X comic book story. Participants were informed that they were to play the role of Professor Thornton, Director of the Weapon-X project, with the job to create the ultimate living weapon, using metallurgic skeletal bonding to convert Logan into Wolverine. Failure results in the feral mutation of Logan. Participants were further informed that in order to learn Thornton's secret, they were to carefully observe his work in order to work out the causes of success (Wolverine) versus failure (the feral mutation).

As for the previous task, the training phase consisted of 45 learning trials, presented as 9 cycles of the 5 stimulus sequences shown in Table 2. However, in the Weapon-X task, 3 stimuli appeared simultaneously: the CI or its absence (represented by an injector syringe), the CS (a block of a certain fictitious alloy), and a distractor (presented as a type of radiation), for a total of 4 seconds on screen. These images were followed by a 1-second presentation of an image of Logan in the midst of the attempted transformation, then the presentation of the US or its absence. The success or failure of the

metallurgical bonding was represented by an image of Wolverine as the UCS, or a picture of feral Logan representing the absence of the UCS (Figure 1). Participants were required to click the mouse to continue. As above, the testing phase consisted of 20 further trials with the key S<sub>g</sub> and CS<sub>t</sub> summation test presentations. The procedure for test trials was identical to that used in training, except that prior to the presentation of the US or its absence, participants were presented with an on-screen rating, scaled 1-9, with a rating of 9 to represent the highest likelihood of success, as in task 1.

### **Design and Analysis**

Analysis of variance (ANOVA) was run in a mixed design with up to four within-subjects factors to assess the development of conditioned inhibition: inhibition (the presence or absence of the CI); task (Mission-to-Mars versus Weapon-X); stimulus type (S<sub>g</sub> vs. CS<sub>t</sub>); presentation, of which there were five levels.

Diagnosis and medication were between subjects factors, examined in successive analyses. The effect of diagnosis was examined overall. The effect of medication was examined within the TS group. The on-medication participants were under treatment with clonidine (n=7) or clonazepam (n=1) at the time of behavioural testing. An off-clonidine group (n=7) were not under treatment with clonidine or clonazepam at the time of behavioural testing, but this group included one participant under treatment with aripiprazole (which has a different mechanism of action, discussed below). Analyses were collapsed across task, stimulus type and presentation where these factors did not affect the development of conditioned inhibition. Planned comparisons (*t*-tests at *p*=0.05) were conducted to examine effects of a priori interest.

The dependent variable to assess conditioned inhibition in the factorial analyses (and shown in Figures 2-3) was the participants' raw expectancy scores (for appearances of an intact rocket in task 1 or the successful transformation of Logan into Wolverine in task 2). In addition, a conditioned inhibition ratio was calculated by dividing the average expectancy score for non-inhibited stimulus presentations by the average expectancy score for inhibited stimulus presentations. Thus conditioned inhibition is indicated by a ratio greater than one and the absence of conditioned inhibition by a ratio less than or equal to one. Thus this derived variable provided a single summary inhibition score per participant, adjusted for the expectancy of the non-inhibited stimulus, which was used in correlational analyses. Specifically, the interrelationship between the level of conditioned inhibition summarized by the ratio and symptom severity scores (measured by the YGTSS) was explored by Pearson's *r* correlation (the total YGTSS scores were available for all participants; the subscale scores were available for all but one participant).

Where the data were available (for 12/15 TS participants), the same analyses were repeated to examine conditioned inhibition performance on each of the tasks in relation to TS participants' IQ. Similarly, the effect of medication was further examined by correlational analysis, using both the raw duration and a ratio to adjust for participants' age (length of time on medication divided by the age of the participant).

## Results

There was a main effect of inhibition ( $F_{1,32}=28.184$ ,  $p<0.001$ ). Figure 2A shows that the TS participants demonstrated an overall equivalent level of inhibition to the matched controls, and this was confirmed statistically in that there was no significant interaction between the diagnosis and inhibition ( $F_{1,32}=0.079$ ,  $p=0.781$ ). Figure 2B shows that performance was equivalent in the two task variants, and statistically there were no significant interactions involving task and inhibition ( $F_{1,32}=1.439$ ,  $p=0.239$ ). Similarly, there were no effects of stimulus type ( $S_g$  vs.  $CS_t$ ) or presentation with respect to inhibition ( $F_{1,32}=3.725$ ,  $p=0.063$ ).

Figure 3 shows that conditioned inhibition was reduced by medication in the TS sample. This impression is confirmed statistically by a significant interaction between medication and inhibition ( $F_{1,13}=5.881$ ,  $p<0.05$ ). Exclusion of the single participant on clonazepam from the on-medication group, primarily composed of participants on clonidine, does not change this conclusion in that the interaction between medication and inhibition remained significant ( $F_{1,12}=5.359$ ,  $p<0.05$ ). The participant on clonazepam is therefore included with the participants on clonidine for the subsequent analyses.

Conditioned inhibition (shown as the difference between non-inhibited and inhibited response scores) was clearly demonstrated in TS participants in the off-clonidine group ( $t_6=4.626$ ,  $p<0.01$ , Cohen's  $d=3.62$ ). By contrast, conditioned inhibition was absent in TS participants who were on medication at the time of testing ( $t_7=1.323$ ,  $p=0.228$ ). Further planned comparisons confirm that the reduction in conditioned inhibition arose primarily because of a relatively large effect of medication in the inhibited condition ( $t_{13}=3.061$ ,  $p<0.01$ , Cohen's  $d=1.7$ ). There was no significant difference in excitatory learning measured in the non-inhibited conditions in relation to medication status ( $t_{13}=1.82$ ,  $p=0.092$ ).

The Mission-to-Mars task was implicit, and procedures were equivalent as far as possible, so learning was not measured during the training phases. Therefore, in order to assess the level to which learning had occurred in the different groups, the data from the first test presentation of the non-inhibited  $CS_t$  (which was used during training phase) were analysed. Univariate analysis of the first test presentation showed no overall significant difference between the TS groups (on- or

off-clonidine) and the matched controls ( $F_{2,31}=1.999$ ,  $p=0.153$ ). Neither was there any difference by task: Weapon-X task ( $F_{2,31}=0.498$ ,  $p=0.612$ ); Mission-to-Mars task ( $F_{2,31}=1.437$ ,  $p=0.253$ ).

### Possible confounds

There was no correlation between symptom severity measured by the YGTSS overall and performance on either of the conditioned inhibition tasks as summarized by the conditioned inhibition ratios (maximum  $r_{14}=0.241$ ,  $p=0.388$ ). There were similarly no significant correlations between conditioned inhibition performance and the level of phonic symptoms (maximum  $r_{14}=0.315$ ,  $p=0.273$ ), motor symptoms (maximum  $r_{14}=0.276$ ,  $p=0.34$ ) or phonic plus motor symptoms total score (maximum  $r_{14}=0.314$ ,  $p=0.274$ ). There was a significant relationship between IQ and the Mission-to-Mars conditioned inhibition ratio ( $r_{12}=-0.606$ ,  $p<0.05$ ), but this was inconsistently demonstrated in that there was no such correlation between IQ and the Weapon-X conditioned inhibition ratio ( $r_{12}=-0.350$ ,  $p=0.264$ ).

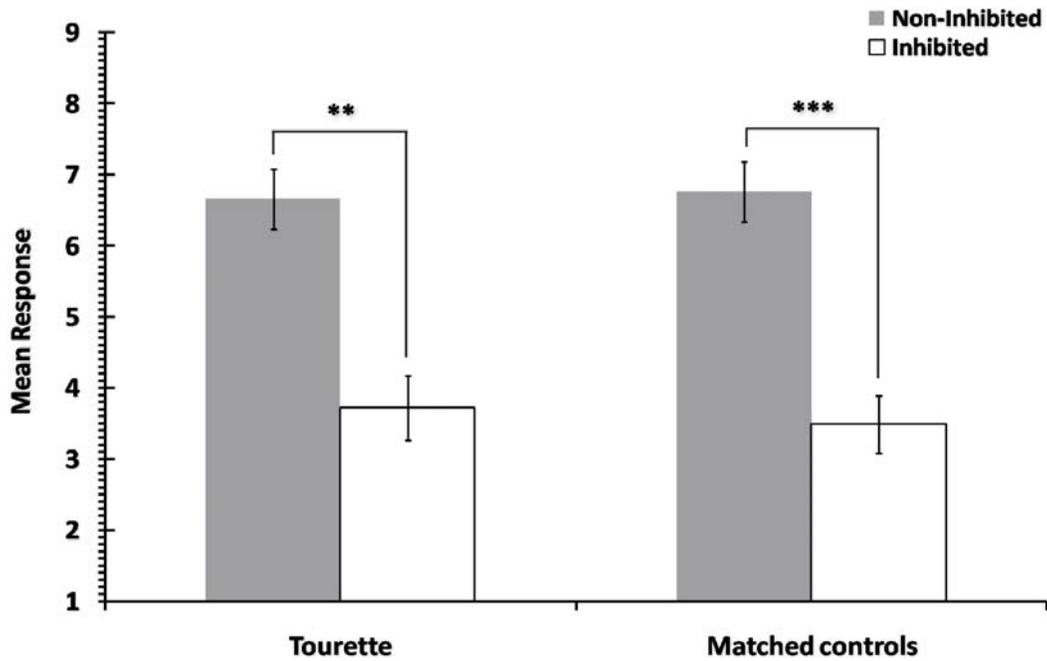
For TS participants in the on-medication group, there was no correlation between performance on the conditioned inhibition tasks and medication duration (as the raw score or adjusted for participants' age; maximum  $r_8=-0.555$ ,  $p=0.153$ ). To further address possible confounds of medication with age (as age may have affected conditioned inhibition) and/or tic severity (as those TS participants on- or off-clonidine may have had a different symptom profile), analyses of covariance were conducted using age and/or scores of the YGTSS as covariates of medication for the TS group. It was found that the significant interaction between inhibition and medication remained intact regardless of the age ( $F_{1,13}=5.46$ ,  $p<0.05$ ) and (total) YGTSS scores of the TS participants ( $F_{1,13}=5.501$ ,  $p<0.05$ ).

However, there were overall sex differences in conditioned inhibition in the sample of TS participants and their matched controls. This was demonstrated statistically as a significant interaction between inhibition and sex ( $F_{1,32}=7.56$ ,  $p=0.01$ ) whereby conditioned inhibition was overall demonstrated by the male participants ( $t_6=6.157$ ,  $p<0.001$ ) but not the females ( $t_6=0.413$ ,  $p=0.694$ ). This difference in conditioned inhibition arose because, compared to the females, the male participants showed greater level of excitatory learning (non-inhibited) responses ( $t_{32}=3.20$ ,  $p<0.01$ ) as well as greater inhibition, shown as a lower level of inhibited responses ( $t_{17.84}=2.886$ ,  $p=0.01$ ).

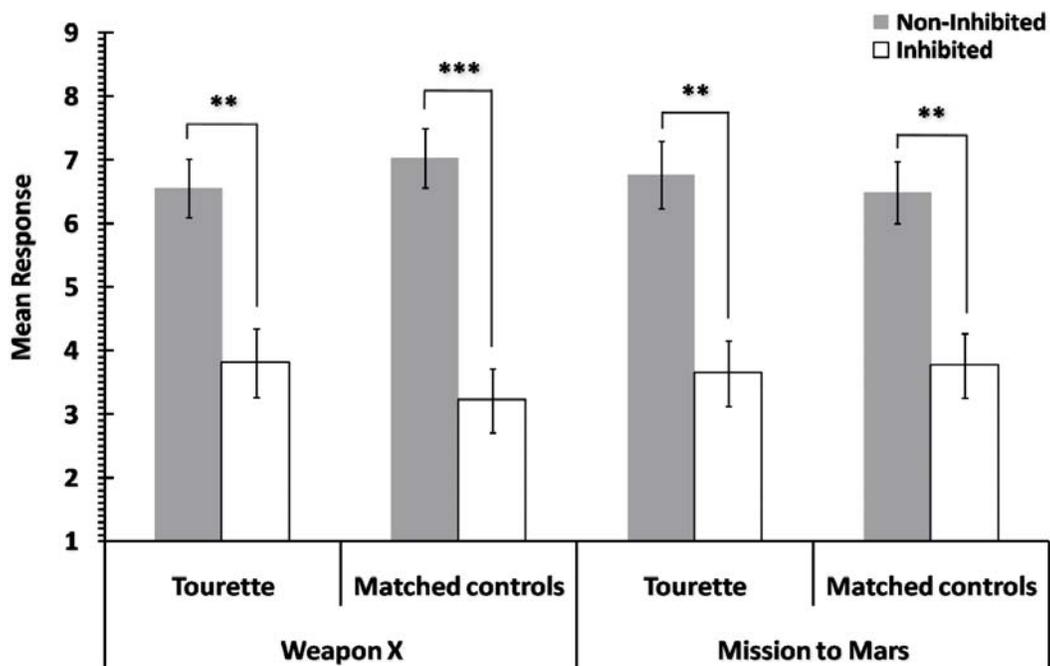
This sex difference might be irrelevant to our overall conclusion in that controls were matched by sex as well as age in order to examine conditioned inhibition in TS. Moreover, there were only 3 female participants with TS and 4 age-matched female controls. However, medication status

**Figure 2. The overall effect of inhibition with respect to diagnostic group overall (A) and by each task variant (B).**

2A



2B



Conditioned inhibition was significant in all groups (\* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ ). Mean Response refers to the participants' expectancy ratings, scaled 1-9, with a rating of 9 to represent the highest likelihood of the outcome (see text for further details).

was determined by factors outside of our control: one female was off- whilst two were on-medication. Thus the sex difference in conditioned inhibition could in principle contribute to the apparent effect of medication. Accordingly, the key analysis was also run with the female participants excluded: the interaction between inhibition and medication remained significant, both with ( $F_{1,10}=7.79$ ,  $p=0.02$ ) and without ( $F_{1,9}=6.40$ ,  $p<0.05$ ) inclusion of the participant on clonazepam.

## Discussion

The learning of conditioned inhibition was confirmed by the summation test for conditioned inhibition (Rescorla, 1969), specifically by the transfer of inhibition to a CS not previously presented with the CI during training ( $CS_t$ ) and to a novel stimulus from the same category, to which participants would generalised their excitatory responding ( $S_g$ ). Statistically, there was no difference in the level of conditioned inhibition demonstrated by stimulus type, or task. The equivalence of results across the two stimulus types shows that the inhibitory properties of the CIs transferred both to a stimulus with which it had never previously been experienced and to a new stimulus with which participants had no explicit prior training of any kind. The tasks were very different in terms of content but formally identical with respect to design and procedure, in all aspects other than the task instructions and the serial versus simultaneous presentation of the CIs. That there was no difference in performance by task suggests that explicit learning instructions are not necessary to show conditioned inhibition with the kinds of procedure in use and that the serial versus simultaneous presentation of the CIs was not an issue.

Counter to prediction, participants with TS showed normal inhibition of stimulus-stimulus associations. Clinically TS has long been viewed as a disorder of inhibition yet experimental studies typically find little deficit on tasks thought to involve inhibitory processes (Channon et al., 2003, 2006, 2009; Roessner et al., 2008). Indeed there is even some evidence of enhanced performance on a saccadic shifting task in TS (Mueller et al., 2006). Typically measures of inhibitory (dys)function have used volitional response measures of the kind Nigg (2000) classifies as effortful, involving conscious control (see also Channon et al., 2009). Our tasks tap a different aspect of inhibitory (dys)function, moreover with no difference between the explicit and the implicit learning variant, thus falling within Nigg's classification of automatic inhibition. Within this category, other studies, for example of inhibition of return (Yuen et al., 2005) and negative priming (Ozonoff et al., 1998) have found no evidence for inhibitory deficits in cases of TS without comorbidity. Thus although the above 'automatic' tasks are very different tasks from the

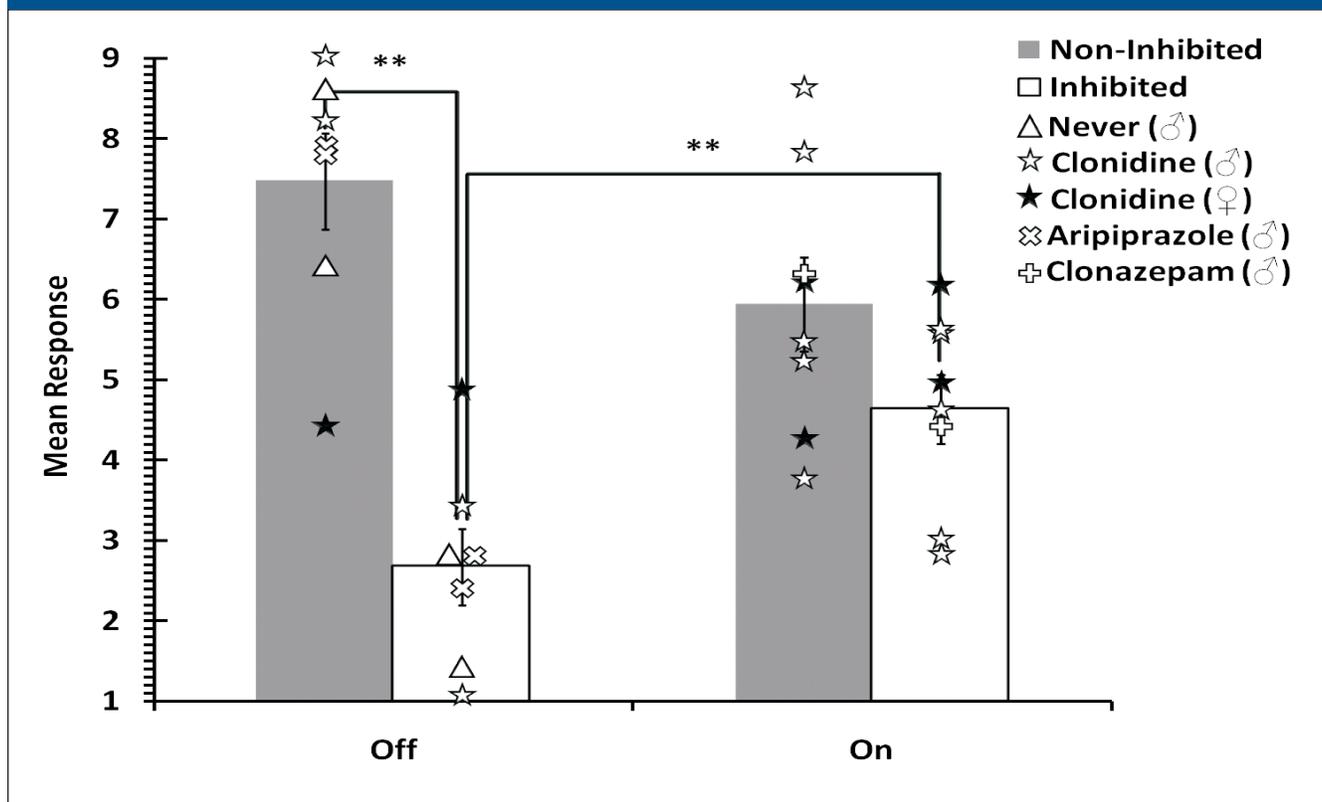
current one, in that they do not rely heavily on learning, the overall pattern of outcomes (mixed results from effortful inhibitory tasks, negative results from more automatic tasks) is broadly consistent with the outcome of the present study of participants with TS without comorbidity.

However, when the participants with TS were examined with respect to their medication, a clear difference was seen in the level of conditioned inhibition. The majority of the participants were medicated with clonidine which has some anxiolytic properties (typically attributed to reduced sympathetic activity; Bremner et al., 1996). The participants treated with aripiprazole (of which only one was currently medicated at the time of behavioural testing) were categorized as 'off-medication' because aripiprazole is an atypical antipsychotic with a different profile of action, which does reduce noradrenergic activity. Whilst aripiprazole has recently been reported to relieve some symptoms of anxiety and depression, this efficacy has only been demonstrated as an adjunctive treatment in the context of residual symptoms that are resistant to treatment with anti-depressants (Adson et al., 2005, Worthington et al., 2005) and has been attributed to the drug's serotonergic mechanisms of action (Pae et al., 2008). The clonazepam participant was included in the on-medication group because of its anxiolytic properties as a benzodiazepine. Although the effects of benzodiazepines on the noradrenergic system are indirect, similar to clonidine, they reduce noradrenergic activity (Gray, 1982; Bremner et al., 1996). As might be expected, the peripheral effects of benzodiazepines, for example their hypotensive properties, are similar to those of clonidine (Hossmann et al., 1980). In any event, analyses excluding the participant with clonazepam produced the same outcome.

From a theoretical perspective, the selective effect on inhibitory as opposed to excitatory learning could be related to clonidine's actions on the Behavioural Inhibition System. According to Gray (1982), reduced noradrenergic activity should impair the processing of signals of nonreward of the kind provided by the CIs used in the present study. The gray frame (task 1) and the injector syringe (task 2) specifically predicted that the rewarding outcome would not occur (represented by the alternate outcomes of an exploded rocket in task 1 and feral Logan in task 2). Such processing was clearly impaired in participants under clonidine. However, the Behavioural Inhibition System was developed as a theory of anxiety rather than conditioned inhibition so this account is only applicable to CIs for rewarding outcomes.

Alternatively, the reduction of sympathetic activity caused by clonidine may result in the disruption of attentional or learning processes necessary to task performance. With respect to learning, we cannot distinguish effects on the acquisition of the inhibitory association during the training phase from

Figure 3. The effect of inhibition in relation to medication.



The individual data points show the sex of the participants ( $\sigma$  = male, open symbols;  $\varphi$  = female, closed symbols) and their medication status. In the 'off' group, three participants had formerly been on clonidine and one had formerly been on aripiprazole, one was still on aripiprazole (see text for explanation), two had never been on any medication. In the on-medication group all of the participants were on clonidine save one who was on clonazepam. Conditioned inhibition was significant in the off- but not in the on-medication group because of a reduction in inhibitory learning in the on-medication participants

\* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

Mean Response refers to the participants' expectancy ratings, scaled 1-9, with a rating of 9 to represent the highest likelihood of the outcome (see text for further details).

effects on the expression of inhibitory learning at test. The earlier acquisition of the discrimination between inhibited and non-inhibited stimuli was not directly assessed in the training phase, because of the implicit nature of the Mission-to-Mars task instructions. However, for both task variants, trial 1 responding to the transfer stimulus provided a measure of the level of excitatory learning, and any differences between groups, before the introduction of a CI for this association. Analysis of excitatory conditioning to the transfer stimulus showed no differences between the diagnostic groups, or by medication in TS participants, in either task. This lack of difference would seem to rule out any obvious impairment of the attentional or learning processes necessary to task performance under clonidine. Nonetheless, the mechanism underlying the modification of the conditioned association in the 'inhibited' condition could be viewed in terms of attention moderating the salience of the different stimuli. For example, the medicated group may not have apportioned sufficient attention to the CI in order for the modification of the conditioned response to occur. It is also possible that there

was a difference in difficulty between the two learning conditions. Rather than inhibiting the excitatory association directly, the 'inhibited' condition could require the formation of more complex learned associations. This alternative explanation is testable in the sense that, if true, clonidine should similarly impair learning in configural learning tasks.

In other words, the underlying mechanism(s) for the difference by drug treatment, and the involvement of inhibition can only be inferred. However, numerous studies with equivalent tasks in animals have shown that the conditioned response is suppressed following concurrent or preceding inhibitory stimuli (Pavlov, 1927, Rescorla, 1969). This suppression of conditioned responding is the basis of the summation test used here. Moreover, although there was no direct measure of what was occurring at a physiological level in the present study, dopamine neurons have been found to show opposite patterns of activity during inhibitory and excitatory conditioning (Tobler et al., 2003).

## Conclusions

Through impaired conditioned inhibition, certain medications for TS could impair potential cognitive control mechanisms for the suppression of tics (via an action on the associative chain that generates triggers). Specifically, impaired inhibition of stimulus-stimulus associations could leave TS sufferers less able to inhibit tics in the presence of the premonitory urge when cues to inhibit are present in the environment. Such contextual factors have recently become the focus of behavioral treatments for TS: through extinction of the excitatory association (Verdellen et al., 2008); and in their capacity as discriminative stimuli in relation to tic consequences (e.g., reinforcement for suppression; Woods et al., 2009). Thus the cognitive side effects of drugs like clonidine should be taken into account in weighing up the costs and benefits of treatments of this kind (Srouf et al., 2008; Tiplady et al., 2005). In particular, the present findings point to the need for specific studies to investigate the moderator effects of clonidine on the efficacy of behaviour therapies for tics.

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