**Antagonism of the D₂ dopamine receptor enhances tremor but reduces voluntary
muscle activation in humans**

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Highlights

- Our knowledge of how D receptors influence movement is mostly based on pathology
- Involuntary and voluntary movement was assessed after haloperidol administration
- Tremor amplitude and muscle activation was compromised with D₂ receptor antagonism
- Movement effects were most prevalent when neural drive to the muscle was high

ABSTRACT

Neural circuits that comprise the indirect pathway in the basal ganglia have been implicated in tremor genesis, and possibly play a role in the voluntary activation of muscles. However, an absence of *in vivo* human studies that target striatal D₂ dopamine receptors of the indirect pathway have prevented causal links being made between the D₂ receptor and motor control. Healthy individuals ingested 3 mg of the competitive D₂ antagonist haloperidol in a double-blinded, placebo-controlled, two-way, cross-over study. Two experiments were performed to examine involuntary and voluntary movement. The first experiment ($n = 10$) assessed time- and frequency-domain measures of force tremor during isometric elbow flexions, and the second experiment ($n = 8$) examined voluntary activation of the elbow flexors during unfatigued and fatigued maximum contractions. Blockade of the D₂ receptor had no effect on tremor frequency, but increased the amplitude of force variability and 8-12 Hz power during moderate intensity isometric elbow flexions. These findings provide direct evidence that D₂ receptors relate to physiological tremor generation during muscle contractions, whereby the gain of tremor is increased after D₂ antagonism. The ability to voluntarily activate the elbow flexors was compromised under both non-fatigued and fatigued conditions. Consequently, the duration that maximum contractions could be sustained was reduced with D₂ antagonism. These results provide further support that the D₂ receptor has a critical role in skeletal muscle activation, where central fatigue is exacerbated by enhancing activity of the indirect basal ganglia pathway during maximum muscle contractions.

Keywords dopaminergic system, maximum voluntary contraction, steadiness, twitch interpolation, central fatigue

1. Introduction

Dopamine is a catecholamine neuromodulator critical for motor control. Within the nigrostriatal pathway, there are two cellular mechanisms to facilitate movement. Pars compacta nigral dopamine projections synapse at striatal Gs-coupled D₁ receptors to depolarize medium spiny neurons (MSNs) in the direct pathway. This, in turn, activates a series of events where motor cortical areas interact with spinal motoneurons to facilitate movement. In contrast, Gi/Go-coupled D₂ receptors located at the striatum promote the hyperpolarization of MSNs, thus further facilitating muscle activity by reducing the inhibitory activity of the indirect pathway (Gerfen et al., 1990; Gerfen and Surmeier, 2011). Presynaptic D₂ auto-receptors also exist at the pars compacta, and not only act to inhibit dopamine neuron excitability, but also dopamine release (Ford, 2014). Therefore, a combination of pre-synaptic and post-synaptic mechanisms exist to carefully regulate movement.

Although motor control is tightly regulated by D₁ and D₂ receptors, our understanding of how these receptors influence movement in humans is poor. Indeed, the majority of our understanding has been inferred from extrapyramidal symptoms observed in clinical populations. For example, typical neuroleptic drugs exert a therapeutic D₂ receptor blockade, and a consequence of using typical neuroleptic agents for extended periods is the emergence of dyskinesia (Haddad et al., 2012). Furthermore, the tremor observed in Parkinson's disease has been linked to the D₂ receptor, where a loss of pre-synaptic pars compacta neurons lead to over-activity in the indirect pathway (Bergman and Deuschl, 2002). Substantial insight into dopaminergic projections have been gained from animal studies, where knock-out mice that lack the D₂ receptor exhibit increased tremor (Bello et al., 2017), and D₂ receptor agonists reduce resting tremor in monkeys after the unilateral lesioning of the ventromedial tegmentum (Goldstein et al., 1973; Kohno et al., 1997). D₂ receptor activity also plays an

important role in regulating tremor activity during voluntary muscle contractions, as the D₂ antagonist haloperidol enhances isometric forelimb tremor when rats perform forelimb pressing tasks (Fowler et al., 1994; Fowler et al., 1990). Collectively, these detailed animal studies indicate that a blockade of the D₂ receptor may compromise the ability to maintain strong isometric muscle contractions.

Given that the dopaminergic system plays a critical role in modulating muscle control, it is surprising that the relationship between nigrostriatal dopamine receptor activity and force generation has received little attention. Of the limited data available, it has been reported that dopamine reuptake inhibition does not affect maximal force production, or CNS activation of fatigued and unfatigued muscle in healthy individuals (Klass et al., 2012). However, it should be noted that reuptake inhibition enhances the availability of endogenous dopamine in the synaptic cleft without necessarily modifying the response of post-synaptic dopamine receptors. Therefore, it cannot be ruled out that dopaminergic systems play a critical role in muscle activation and neuromuscular fatigue. It is likely that the antagonism of dopamine receptors in healthy individuals may reveal changes in voluntary muscle control. Animal models support this notion, where a D₂ receptor blockade suppresses treadmill running (Ahlenius and Hillegaart, 1986; Hillegaart and Ahlenius, 1987; Hillegaart et al., 1987), and the destruction of nigrostriatal dopamine neurons impairs time to exercise exhaustion in rats (Derevenco et al., 1986; Heyes et al., 1985). This suggests that a drug-induced 'overactivity' of the indirect pathway of the basal ganglia may compromise voluntary muscle activation and contribute to the development of neuromuscular fatigue.

The purpose of this study was to determine how the D₂ dopamine receptor influences involuntary and voluntary human movement. Two experiments were performed where the D₂ antagonist haloperidol was used to induce a reversible blockade of the D₂ receptor in the nigrostriatal pathway. In our first experiment, we assessed force tremor during isometric

elbow flexions at 10% and 60% maximum voluntary contraction (MVC) using time- and frequency-domain techniques. In our second experiment, we examined voluntary activation for unfatigued and fatigued maximum elbow flexions via twitch interpolation. We hypothesised that a blockade of the D₂ dopamine receptor would (i) enhance the amplitude of force tremor without altering the frequency of tremor, (ii) decrease voluntary activation in unfatigued muscle, and (iii) decrease voluntary activation to a greater extent than a placebo in the presence of neuromuscular fatigue.

2. Materials and method

2.1 Experiment design

This study was a human, double-blind, placebo-controlled, two-way crossover design with repeated measures. Each participant attended two testing sessions separated by 10 days. In the first experiment, a standardised haloperidol or placebo capsule was administered to each participant at midday ($n = 10$, before each testing session), where capsule administration was counterbalanced to avoid order effects. From this, assessments of force tremor were performed 2 hr after the ingestion of each capsule. In the second experiment, within the same testing session, eight participants also took part in a contraction protocol that assessed voluntary muscle activation after the force tremor tasks.

2.2. Participants

Ten neurologically normal participants (age 22.1 ± 1.8 years, height 176.9 ± 8.2 cm, weight 77.8 ± 12.6 kg, 2 females, all right handed) with no recent history of musculoskeletal injury volunteered to participate in up to two experiments. Participants were screened using a medical history questionnaire that identified any contraindications and safety concerns regarding haloperidol, percutaneous electrical stimulation, and the experimental exercise

task. Participants were asked to refrain from any CNS stimulants or depressants such as caffeine, alcohol, or moderate to high intensity exercise for 6 hr prior to testing. All of the participants were naïve to psychoactive medications. Institutional ethics were obtained for the study, and all procedures were performed in accordance with the Declaration of Helsinki. Written informed consent was obtained prior to the commencement of any testing procedures.

2.3. Drug intervention

A single 3 mg dose of haloperidol was administered orally. This titration provided a safe way of assessing acute motor changes in healthy individuals, without compromising participant safety by inducing serious extrapyramidal reactions. Both the haloperidol and placebo pills were compounded in opaque capsules to ensure double-blinding for the drug condition. The recommended therapeutic dose of haloperidol is 1-15 mg per day in adults, although it may be increased up to and above 100 mg in order to control clinically specific symptoms. The antagonistic effect of haloperidol on post-synaptic D₂ receptors is well established, and hence functions as an anti-dopaminergic agent within the CNS (Naiker et al., 2006; Osinski et al., 2005; Seeman, 2010; Uchida et al., 2007). Although the peak plasma concentration of haloperidol has a large window (estimated to be between 2 and 6 hr), pilot testing revealed that there were movement effects at approximately 2 hr post-ingestion. Hence, the assessment of movement for all participants occurred 2 hr post-ingestion, as it was assumed that the drug had crossed the blood-brain barrier and started to interact with dopamine receptors.

2.4. Elbow flexion torque

All measurements obtained in this study were performed on the participant's dominant arm using the same custom designed transducer (Fig. 1A). An aluminium device positioned the forearm vertically with the elbow in 90 degrees of flexion. A firm-back adjustable chair permitted 90° flexion at the shoulder while the arm was secured firmly into the device at the wrist with a non-compliant strap. A calibrated PT4000 S-Type tension and compression load cell (200 kg, PT Ltd., NZ) was attached to the posterior side of this aluminium device to assess elbow flexion force. From this, raw elbow flexion force could be converted to elbow flexion torque based on the distance between the lateral epicondyle and the point of force application at the wrist ($\text{torque} = \text{force} \times \text{lever arm}$). All force data was sampled at 2000 Hz using a Power 1401 interface with Spike2 software (version 7, Cambridge Electronic Design Ltd., UK).

2.5. Electromyography

Surface electromyography (EMG) was obtained from the biceps brachii and triceps brachii using bipolar 24 mm Ag/AgCl electrodes with an inter-electrode distance of 24 mm (Kendall ARBO). Prior to electrode attachment, the underlying skin was shaved, abraded and cleaned using an ethanol solution and soft gauze. A reference electrode was placed over the acromion of the test limb. Surface EMG signals were differentially amplified ($\times 1000$) by a NL844 pre-amplifier, bandpass filtered (10 Hz - 500 Hz) by a NL135 Low Pass Filter and NL144 High Pass Filter (all Digitimer Ltd., UK) and sampled (2000 Hz) via a Power 1401 interface with Spike2 software (version 7, Cambridge Electronic Design Ltd., UK).

2.6. Motor nerve stimulation

The intramuscular nerve fibres innervating the elbow flexors were stimulated with single supramaximal electrical pulses of 100 μs duration via a constant current nerve

stimulator (DS7AH, Digitimer Ltd., UK). A surface anode was positioned over the distal biceps tendon, and a surface cathode was positioned between the elbow crease and the most anterior edge of the deltoid (overlying the biceps). The optimal cathode position for each participant was determined via a motor point stimulating pen. The pen was used to find the location that elicited the highest elbow flexor twitch torque whilst minimising elbow extensor activation. After the optimal stimulating position was identified, a surface electrode was placed over that site for subsequent test stimulations. A maximal resting twitch was quantified by gradually increasing the stimulation intensity until there were no further increases in peak torque generated by the elbow flexors. The stimulus intensity used in the study was fixed at 20% above the maximal resting twitch intensity (96-456 mA).

2.7. Experiment protocols

Prior to experimental testing, participants were familiarised with the apparatus and practiced maximal elbow flexion while attached to the device. Submaximal isometric contractions were not practiced as this can reduce the amplitude of force tremor which may influence the outcomes of the study. Each participants baseline MVC was determined at the commencement of both testing sessions (Fig. 1B). For *Experiment 1*, five brief maximum effort isometric contractions (~2 s) were performed with a 3-4 min rest period between each contraction. Within each session, the contraction with the largest torque was considered the participants MVC. Standardised verbal encouragement was provided to ensure each participant was contracting at maximum torque.

After establishing baseline MVC strength, force tremor was assessed during isometric elbow flexions at 10% MVC and 60% MVC. Three 10 s trials were collected for each intensity. The target elbow flexion force, and the force that the participant was generating, were presented on a PC monitor positioned 1 m in front of the participant at eye level. As

visual feedback gain is known to affect force tremor amplitude and targeting error (Kenway et al., 2015; Slifkin et al., 2000; Tracy et al., 2007; Vaillancourt and Russell, 2002), the scale of the y-axis of the force trace was standardized between contraction intensities and testing sessions. The y-axis scale was made relative to the peak MVC during that session, and the range above and below the target force was the same magnitude as the calculated target force. For example, if a participant's target force was 40 N, then the y-axis would display a range of 0-80 N.

For *Experiment 2*, the voluntary activation characteristics of the elbow flexors were assessed. During five brief isometric MVCs, a single electrical stimulation was delivered to the elbow flexors to elicit a superimposed twitch. Four seconds after each maximum contraction, a single electrical stimulation was delivered to the elbow flexors at rest so that a potentiated resting twitch was obtained. From this, an isometric contraction protocol was employed to induce elbow flexion fatigue, whereby 6 sustained maximum contractions were performed until torque declined to 60% MVC for 3 s. These sustained maximum contractions were followed 3 s later by a resting twitch, and then 3 s later by a brief maximum contraction with a superimposed twitch. The timing of the superimposed twitch reduced the likelihood that central mechanisms of fatigue had recovered following the fatiguing contraction. After the cessation of the brief maximum contraction, a strict 90 s rest period occurred in which a measure of perceived elbow flexor fatigue was obtained for the previous sustained fatiguing contraction. Ratings of perceived elbow flexor fatigue were measured for each participant using a CR10 Borg Rating of Perceived Exertion (RPE) scale (Borg, 1982). Participants were instructed that a '0' indicated a 'sensation of the arm at complete rest', and a '10' meant that it 'would be impossible to even lift the fatigued arm'.

2.8. Data analysis

Tremor characteristics of each participant were profiled by examining the 10% MVC and 60% MVC isometric contractions in the time- and frequency-domain. The coefficient of variation (CV) of force was calculated during the middle 8 s of each 10 s isometric contraction. The CV is a normalized measurement of variability in the force signal ($CV = \text{standard deviation of force} / \text{mean of force} \times 100$), whereby a higher CV represents greater force variability regardless of changes in contraction intensity (i.e. 10% MVC compared to 60% MVC). The same 8 s epochs of force data were examined in the frequency domain by extracting power spectral content using Welch's averaged periodogram method. The Fast Fourier Transform (FFT) was set at 4096, which resulted in a frequency resolution of 0.479 Hz. The peak power between 8-12 Hz and the frequency of peak power were extracted from the power spectra of each contraction. The 8-12 Hz frequency bandwidth represents isometric force power and frequency associated with physiological tremor in neurologically normal individuals. Data was averaged for the 3 trials at 10% and 60% MVC. All force tremor data analysis was performed offline using Spike2 (version 7, Cambridge Electronic Design Ltd., UK).

All force data collected during the fatigue protocol, including the unfatigued MVCs, was converted to torque. Time to fatigue, the area under the elbow flexion torque curve, and the area of biceps EMG were calculated for each sustained maximal contraction. These measurements were calculated from the onset of elbow flexion torque to the time that torque declined to 60% MVC for greater than 3 s using built-in Spike2 functions. Elbow flexion torque and biceps EMG area relate to the amplitude and duration of the sustained elbow flexion, both of which are factors that contribute to motor fatigue. For the brief MVC trials, the peak-to-peak superimposed twitch and resting twitch torque values were extracted within each session pre- and post-fatigue. The peak-to-peak amplitude of the superimposed twitch and resting twitch were calculated from increases in the torque signal due to the electrical

stimulation. The twitch values were then used to calculate the level of voluntary activation (%) for each participant via the equation: $(1 - \text{superimposed twitch}/\text{resting twitch}) \times 100$. All twitch data was exported to Signal (version 6, Cambridge Electronic Design Ltd., UK) for offline analysis.

2.9. Statistical analysis

A paired samples *t* test was conducted to determine if there were baseline differences between the placebo and haloperidol (unfatigued) MVC torque. Two-factor repeated measures ANOVA were used for all comparisons in this study. For force tremor, the effect of drug (placebo, haloperidol) and contraction intensity (10% MVC, 60% MVC) was examined for CV of force, peak 8-12 Hz power, and the frequency that the peak 8-12 Hz power occurred. For the sustained contraction protocol, the effect of drug (placebo, haloperidol) and individual contraction (contraction 1-6) was examined for time-to-fatigue, elbow flexion torque area, biceps EMG area and ratings of perceived elbow flexor fatigue. In addition, the effect of drug (placebo, haloperidol) and overall fatigue (pre, post) was examined for the measures of superimposed twitch torque, resting twitch torque, and level of voluntary activation. For ANOVA, Mauchly's test of sphericity was applied to each variable and Greenhouse-Geisser corrections were used if sphericity was violated. Fisher's Least Significant Difference (LSD) post hoc tests were employed to reveal within and between factor differences. All of the data presented in text and figures are represented as group means $\pm$ SD. All statistical tests were performed using SPSS Statistics (version 24, IBM Corp., USA), and statistical significance was set at $p < 0.05$.

3. Results

All participants tolerated the dose of haloperidol well. Although there were no reported adverse effects, and no participant could distinguish which pill they had ingested, some participants subjectively reported that they felt ‘unusually tired’ in the hours following the testing session. This tiredness typically subsided ~3 hr after the completion of testing.

3.1. Baseline MVC torque

Peak elbow flexor MVC torque was measured in unfatigued muscle following drug administration. MVC torque was significantly different between drug conditions, where MVC torque 2 hr post-ingestion of haloperidol (76.93 ± 22.25 Nm) was lower than the placebo condition (81.23 ± 21.64 Nm) ($t(9) = 2.29$, $p = 0.024$). As there were no differences between baseline measurements in *Experiment 1* and 2, only MVC for the first experiment is reported here.

3.2. Force tremor

Participants performed isometric elbow flexions at 10% MVC and 60% MVC in *Experiment 1*, whereby time- and frequency-domain analyses of tremor were extracted from an 8 second window during the contraction. Fig. 2 illustrates force tremor data for a single participant during the 10% MVC and 60% MVC tasks. Overall, participants exhibited larger fluctuations in force after the administration of haloperidol, with distinct increases in the peak 8-12 Hz power observed most notably at 60% MVC.

A main effect of drug was detected for CV of force ($F(1,9) = 5.99$, $p = 0.037$), where CV for the haloperidol condition was greater than the placebo condition (Fig. 3A). Planned comparisons revealed that the CV of force for the haloperidol condition was greater than the

placebo condition at 60% MVC ($p = 0.015$), but not 10% MVC. A main effect of contraction intensity was also identified ($F(1,9) = 30.74$, $p < 0.001$), where CV of force for the 60% MVC was greater than the 10% MVC condition. Planned comparisons revealed that CV of force at 60% MVC was greater than CV of force at 10% MVC for the haloperidol ($p < 0.001$) and placebo conditions ($p = 0.009$).

In the frequency domain, haloperidol did not change the dominant tremor frequency at 10% MVC (placebo: 9.69 ± 0.78 Hz; haloperidol: 9.85 ± 0.99 Hz) or 60% MVC (placebo: 10.37 ± 0.7 Hz; haloperidol: 10.63 ± 0.88 Hz). A main effect of contraction intensity was identified ($F(1,9) = 9.05$, $p = 0.015$), where the frequency of peak power for the 60% MVC was greater than the 10% MVC condition. Planned comparisons revealed that the frequency of peak power at 60% MVC was greater than frequency of peak power at 10% MVC for the haloperidol ($p = 0.033$) and placebo conditions ($p = 0.015$).

The amplitude of power of the dominant tremor frequency was affected ($F(1,9) = 9.88$, $p = 0.012$), where the peak power for the haloperidol condition was greater than the placebo condition (Fig. 3B). Planned comparisons revealed that the 60% MVC haloperidol condition was greater than peak power for the 60% MVC placebo condition ($p = 0.012$). There was no difference between the 10% MVC haloperidol and placebo conditions for the dominant tremor power. A main effect of contraction intensity was identified ($F(1,9) = 29.95$, $p < 0.001$), where the peak power for the 60% MVC was greater than the 10% MVC. Planned comparisons revealed that peak power for 60% MVC was greater than the peak power for 10% MVC for the haloperidol ($p = 0.001$) and placebo conditions ($p < 0.001$). There was also an interaction effect of drug and contraction intensity ($F(1,9) = 9.66$, $p = 0.013$).

3.3. Time to fatigue, torque and EMG area when performing sustained MVCs

Experiment 2 assessed voluntary activation characteristics following fatiguing maximum elbow flexions. A main effect of drug was detected for elbow flexion time to fatigue ($F(1,6) = 7.02, p = 0.038$) and torque area ($F(1,6) = 7.6, p = 0.033$) where these measurements were reduced for the haloperidol condition compared to the placebo condition. There was no main effect of drug for the biceps EMG area. Although there was an overall effect of haloperidol on the ability to perform sustained maximum contractions, planned comparisons revealed that these effects were most noticeable during the first sustained MVC ($p = 0.045$ for time-to-fatigue, Fig. 4A; $p = 0.01$ for torque area, Fig. 4B). Fig. 4C shows the results for biceps EMG area.

There was a main effect of contraction for elbow flexion time to fatigue ($F(2.27,13.62) = 16.23, p < 0.001$), torque area ($F(2.22,13.32) = 19.95, p < 0.001$) and biceps EMG area ($F(1.55,9.28) = 10.51, p = 0.006$) where these measures reduced from the first sustained MVC onwards. The effect of contraction was most evident early on in the fatigue task, as planned comparisons revealed that the first sustained contraction was longer in duration than the second ($p = 0.009$ for time to fatigue, $p = 0.004$ for torque area, $p = 0.009$ for EMG area), and the second sustained contraction longer than the third ($p = 0.04$ for time to fatigue, $p = 0.016$ for torque area, $p = 0.04$ for EMG area).

3.4. Rating of perceived fatigue during sustained MVCs

Although haloperidol did not affect the participant's RPE during the fatigue task, there was a main effect of contraction ($F(1.35,9.43) = 49.15, p < 0.001$, Fig. 5). In particular, the RPE for elbow flexor fatigue significantly increased between each successive contraction throughout the fatigue protocol (all p 's < 0.006).

3.5. Level of voluntary activation

Fig. 6 illustrates superimposed and resting twitches for a single participant during the performance of fatigued and unfatigued maximum elbow flexions. In general, the superimposed twitch was greater for the haloperidol condition for both the pre-fatigue and post-fatigue trials, whilst resting twitch amplitude was unchanged with haloperidol.

A main effect of drug was observed for superimposed twitch amplitude ($F(1,7) = 56.16, p < 0.001$), where superimposed twitch for the haloperidol condition was greater than the placebo. Planned comparisons revealed that the haloperidol condition was greater than the placebo condition pre-fatigue ($p = 0.005$, Fig. 7A) and post-fatigue ($p < 0.001$). There was also a main effect of fatigue ($F(1,7) = 8.55, p = 0.022$), where superimposed twitch pre-fatigue was less than post-fatigue. Planned comparisons revealed that pre-fatigue was less than post-fatigue in the placebo condition ($p = 0.034$) and the haloperidol condition ($p = 0.020$).

Although resting twitch torque remained unchanged between the placebo and haloperidol conditions, a main effect of fatigue ($F(1,7) = 23.33, p = 0.002$) indicated that pre-fatigue resting twitch torque was significantly greater than post-fatigue. Resting twitch torque decreased with fatigue in both the placebo ($p = 0.002$, Fig. 7B) and haloperidol conditions ($p = 0.005$).

When the superimposed twitch and resting twitch were used to calculate the level of voluntary activation, a main effect of drug was observed ($F(1,7) = 24.93, p = 0.002$). Overall, voluntary activation for the haloperidol condition was lower than the placebo. Planned comparisons revealed that the haloperidol condition was lower than the placebo condition pre-fatigue ($p = 0.007$, Fig. 7C) and post-fatigue ($p = 0.024$). There was also a main effect of fatigue ($F(1,7) = 35.48, p = 0.001$), where pre-fatigue was significantly greater than post-fatigue. In the placebo condition, pre-fatigue was significantly greater than post-fatigue ($p <$

0.001), and in the haloperidol condition, pre-fatigue was significantly greater than post-fatigue ($p = 0.003$).

4. Discussion

The purpose of this study was to determine how the D₂ dopamine receptor influences force tremor and voluntary muscle activation in healthy participants. In our first experiment, we assessed force tremor during isometric elbow flexion at 10% and 60% MVC in the time- and frequency-domain. In our second experiment, we examined voluntary activation for unfatigued and fatigued maximum elbow flexions via twitch interpolation. The results indicate that a D₂ receptor blockade: (i) increased the CV of force and 8-12 Hz power at 60% MVC without affecting the frequency characteristics of tremor, (ii) decreased MVC torque and voluntary activation of the unfatigued elbow flexors, and (iii) induced a greater level of central fatigue during sustained maximum elbow flexions compared to placebo.

4.1. Force tremor is enhanced after antagonism of the D₂ receptor with haloperidol

The antagonism of the D₂ receptor with haloperidol caused an increase in CV of force when performing isometric muscle contractions. Furthermore, haloperidol increased power in the 8-12 Hz bandwidth (which reflects CNS processes; Elble and Randall, 1976), indicating that the enhanced force tremor amplitude could be attributed to neural processes rather than mechanical factors such as enhanced rigidity (which is often observed in basal ganglia disorders) (Jankovic, 2008). Given that drug-dependent changes in tremor occurred during the 60% MVC tasks and not 10% MVC tasks, it appears that D₂ receptors play an important role in regulating tremor amplitude when voluntary drive to the contracting muscle is high. It is likely that the D₂ antagonism with haloperidol influenced the input-output properties of the basal ganglia, in turn enhancing the gain of tremor at 60% MVC.

The generation of tremor is complex, and changes in force tremor may be mediated from several sources in the CNS. For skeletal muscle to contract, both cortical and subcortical structures interact with spinal motoneurons to regulate the amount of activity that occurs at the muscle (Heckman and Enoka, 2012). Although spinal circuits modulate muscle activity, it is unlikely that haloperidol caused a direct change in spinal motoneurone output. Dopamine receptors are expressed on spinal motoneurons in mammals (e.g. in mice) (Zhu et al., 2007), however the direct dopaminergic action on spinal motoneurons has typically been linked to activity at D₁-like receptors, and not D₂-like receptors (Han and Whelan, 2009; Schwarz and Peever, 2011). Therefore, a blockade of D₂ receptors will influence CNS oscillatory activity at locations upstream of the spinal motoneurone pool, in which indirectly modulates the synaptic input to spinal motoneurons. At 10% MVC, low threshold motor units are recruited to perform the isometric contraction. As there was no significant effect of haloperidol at 10% MVC, it might be speculated that D₂ receptor antagonism had minimal effect on the supraspinal inputs to the motoneurone pool that regulate low threshold motor units. Alternatively, the 60% MVC task is a more challenging isometric control task, and a range of low and higher threshold motor units are recruited. Together, these results suggest that haloperidol had the greatest effect on tremor amplitude when a large number of descending inputs to the spinal motoneurons have been activated.

As power in the tremor signal increased without any change in the dominant tremor frequency (~10 Hz), it appears that a single dose of haloperidol enhanced physiological tremor rather than mimic pathological tremor (e.g. 3-6 Hz tremor). Although it seems logical to relate D₂ receptor antagonism to extrapyramidal movement disorders such as Parkinson's disease, Huntington's disease or tardive dyskinesia, our results suggest that such chronic pathological tremors are more complex than tremors induced from single drug doses. In Parkinsonism, a progressive nigrostriatal dopamine neuron loss reduces the pre-synaptic

release of dopamine (Bergman and Deuschl, 2002). However, our study most likely induced a D₂ receptor blockade at post-synaptic sites, meaning that dopamine release from pre-synaptic nerve terminals was not impaired in our healthy experimental sample. In contrast, with movement disorders such as Huntington's disease there is a loss of post-synaptic striatal GABAergic neurons that express dopamine receptors (Albin et al., 1989; Berardelli et al., 1999). As such, the administration of haloperidol would best relate to that of a Parkinsonian pathophysiology. We propose that pathological changes in tremor (like Parkinsonian tremor) do not emerge solely because of reductions in the binding of dopamine to isolated dopamine receptor subtypes, but relate to widespread alterations in supraspinal motor circuits.

To the best of our knowledge, this is the first tremor study in humans that has used a pharmacological intervention to target the D₂ receptor. Overall, our results overlap with findings from animal models. For example, a D₂ receptor antagonism with haloperidol enhances tremor during submaximal isometric forelimb pressing tasks (Fowler et al., 1994; Fowler et al., 1990). Furthermore, there is an enhanced tremor in knock-out mice that lack the D₂ receptor, with these animals showing distinct changes in basal ganglia oscillatory activity (Bello et al., 2017). Pharmacological work performed in monkeys also indicate that the activation of the D₂ receptor (with talipexole or trivastal, both D₂ receptor agonists) reduces resting tremor after the unilateral lesioning of the ventromedial tegmentum (a supraspinal projection area with distinct dopaminergic cell bodies) (Goldstein et al., 1973; Kohno et al., 1997). Collectively, animal and human experiments indicate that the antagonism of D₂ receptors associated with the indirect basal ganglia pathway has a pronounced effect on the control of submaximal isometric muscle contractions, and a subsequent 'overactivity' of indirect pathway circuits enhances physiological tremor. It appears that the D₂ receptor could be a viable pharmacological target for pathological tremors involving the dopaminergic system. Nevertheless, dedicated clinical research needs to be undertaken in this area.

4.2. Blockade of the D₂ receptor decreases voluntary muscle activation during MVCs

Experiment 1 addressed the control of submaximal isometric contractions, where the higher intensity contractions (60% MVC) were compromised with a D₂ dopamine receptor blockade. Maximum elbow flexions could not be assessed in *Experiment 1* because a MVC cannot be maintained at an isometric steady state for any period of time. As such, a second experiment was performed that utilised twitch interpolation at the elbow flexors to determine if the ability to perform maximum contractions are compromised with a D₂ receptor blockade.

The ability to maximally activate muscle is commonly assessed via twitch interpolation, which typically involves the delivery of a single supramaximal stimulation to a muscle during an isometric MVC (Allen et al., 1998; Merton, 1954). If additional force can be generated with stimulation, it means that not all motor axons have been recruited (or are ‘maximally’ firing) during the MVC (Belanger and McComas, 1981; Merton, 1954). In the current study, baseline MVC torque was reduced with haloperidol, which corresponded to an increased superimposed twitch amplitude and reduced level of voluntary activation of the elbow flexors. These findings provide further evidence that the central dopaminergic control of movement was compromised under conditions where strong neural drive is directed towards the target muscle. The nigrostriatal dopaminergic system dis-inhibits the motor cortex via interactions with basal nuclei. As such, a blockade of striatal D₂ receptors most likely enhanced the inhibitory effect of the basal ganglia on motor cortical areas, thus causing a decreased voluntary drive to the motoneurone pool during baseline MVCs (Albin et al., 1989; Alexander and Crutcher, 1990).

There has only been a single study that has assessed MVC performance and voluntary muscle activation after the pharmacological manipulation of the dopaminergic system in

humans. Klass et al. (2012) administered the dopamine reuptake inhibitor, methylphenidate, to well-trained men. The prevention of dopamine reuptake at pre-synaptic nerve terminals had no effect on peak MVC torque or voluntary activation for the unfatigued knee extensors. However, the current study found that the antagonism of D₂ receptors reduced MVC and voluntary activation for the unfatigued elbow flexors. Collectively, these results suggest that increasing intra-synaptic dopamine concentrations does not necessarily enhance the ligand binding effect of dopamine at post-synaptic D₁ or D₂ receptors to facilitate motor performance. Instead, a pharmacological blockade of dopamine receptors is required to induce motor changes, which thereby implicates dopamine receptor antagonism to motor performance as opposed to increases in intra-synaptic dopamine concentrations.

4.3. Enhancing activity in the indirect basal ganglia pathway augments central fatigue

Human motor fatigue has both a central (nervous system) and peripheral (skeletal muscle) component (Gandevia, 2001). Central fatigue was induced via sustained maximum elbow flexions, where a decline in the level of voluntary activation was observed post-fatigue for both the placebo and haloperidol conditions. Although voluntary activation was affected with fatigue, the magnitude of effects was exacerbated during the haloperidol condition. In particular, voluntary activation for the placebo condition reduced by ~9 % in the presence of fatigue, whereas voluntary activation for the haloperidol condition (compared to unfatigued placebo measures) reduced by ~14 %. Both the placebo and haloperidol conditions exhibited a decreased resting twitch with fatigue, and thus, there was some degree of peripheral fatigue for both conditions. However, the post-fatigue resting twitch remained unaffected by the drug. As haloperidol is typically indicated in psychotic disorders, the psychophysical effects of the drug intervention should not be discounted. However, as there was no change in fatigue-related RPE between the placebo and haloperidol conditions, any such influences during the voluntary contractions were minimal.

Given that haloperidol has an antagonistic action towards D₂ receptors, it is possible that there was an atypical loss of inhibition of the indirect basal ganglia pathway during the elbow flexion fatigue protocol. As such, dopamine would be less likely to bind to the D₂ receptor to inhibit the indirect pathway of the basal ganglia. The basal ganglia would therefore be less able to dis-inhibit the motor cortex, causing a decreased drive to the elbow flexors and a premature onset of fatigue (Foley and Fleshner, 2008). Animal models utilising anti-dopaminergic interventions directly support the fatigue related results from this study, where it has been demonstrated that a D₂ receptor blockade with both haloperidol and raclopride suppress treadmill running in rodents (Ahlenius and Hillegaart, 1986; Hillegaart and Ahlenius, 1987; Hillegaart et al., 1987). It has also been shown that the administration of the neurotoxin 6-hydroxydopamine (6-OHDA), which would result in less nigrostriatal dopamine and a decreased activity of dopamine at striatal dopamine receptors, impairs rodent running and swimming time to fatigue (Derevenco et al., 1986; Heyes et al., 1985). Of these studies, Heyes et al. (1985) also highlighted that the administration of the D₂ agonist apomorphine to 6-OHDA lesioned rats not only restored exercise capacity, but prolonged running time to exhaustion on a treadmill. The combined findings from animal studies and our present study highlight the importance of nigrostriatal dopamine neurotransmission during fatiguing exercise tasks, and suggest a role of dopamine in central fatigue.

In general, it is not only believed that reductions in CNS dopamine release induce neuromuscular fatigue quicker, but the pharmacological enhancement of dopamine release may prolong fatigue (Foley and Fleshner, 2008; Meeusen et al., 2006; Taylor et al., 2016). In humans, several studies have attempted to enhance dopaminergic neurotransmission to improve exercise performance during fatiguing exercise tasks (Klass et al., 2012; Meeusen et al., 1997; Onus et al., 2016; Piacentini et al., 2004; Roelands et al., 2008; Swart et al., 2009; Watson et al., 2005; Williams and Thompson, 1973). However, of these human studies, the

overall finding appears to be that enhanced intra-synaptic dopamine concentrations (via dopamine reuptake inhibition) or synthesis (L-dihydroxy-phenylalanine, L-DOPA administration) has a limited effect on motor performance. Conversely, in animal models, dopamine reuptake inhibition and dopamine receptor activation appears to have an ergogenic effect on time to exercise exhaustion, but yet, still offers some conflicting results (Bhagat and Wheeler, 1973; Bracken et al., 1988; Gerald, 1978; Heyes et al., 1985; Kalinski et al., 2001; Snider et al., 1983). As such, it appears that animal models, at least partially, support the notion that enhancing dopamine neurotransmission benefits fatiguing exercise performance. Overall, the targeted antagonism of D₂ receptors enhanced human central fatigue in this study, and these results reiterate the fact that enhancing dopamine availability in humans may not be adequate to reveal changes in fatiguing motor activity. Future human fatigue studies should utilise dopaminergic interventions that act to antagonise dopamine receptors, and not simply rely on increasing the amount of dopamine in the synapse.

4.4. Considerations

Haloperidol is a known D₂ receptor antagonist, but we must acknowledge that haloperidol can also effect non-dopaminergic neurotransmitter systems (Cobos et al., 2007; Gallagher et al., 1998; Gu et al., 2007; Kroeze et al., 2003; Schotte et al., 1993). However, it has been established that the characteristic extrapyramidal movement effects of haloperidol (and other typical neuroleptics) typically relate to its occupancy of and pharmacological action at the D₂ receptor (Farde et al., 1992; Sykes et al., 2017). It is difficult to entirely predict the magnitude of our 3mg haloperidol intervention on D₂ receptor activity. However, in healthy men, it has been shown that a single 4 mg oral dose of haloperidol can induce a D₂ receptor blockade of 73% 3 hr post ingestion (as determined by PET imaging with [¹¹C]raclopride antagonism of the D₂ receptor) (Nordstrom et al., 1992). This D₂ receptor

occupancy also resulted in a diminished radioactivity at the putamen, thus indicating a marked reduction in striatal activity. As such, our 3 mg haloperidol intervention would have likely been sufficient to antagonise a majority of striatal D₂ receptors, a consideration that is supported by the motor effects exhibited in our study.

Whilst it is plausible to relate movement dysfunction to the antagonism of post-synaptic striatal D₂ receptors, it should not be assumed that this is the only CNS location, or receptor, that haloperidol acted upon. For example, it is difficult to preclude the antagonism of pre-synaptic D₂ auto-receptors with haloperidol, in which would increase dopamine production and release from pars compacta nigral neurons. Nonetheless, in the presence of acute increases in synaptic dopamine, haloperidol will still act at post-synaptic D₂ receptors to prevent the further binding of dopamine. Similarly, if an enhanced dopaminergic activity resulted in an increased binding of dopamine to striatal D₁ receptors, this would have a facilitative effect on movement, and in the context of our results, may suggest that the antagonism of the striatal D₂ receptor was more influential in inducing the observed negative motor changes. Future research should seek to isolate relevant supraspinal and/or spinal motor changes after the administration of haloperidol, or other anti-dopaminergic agents targeting specific D₁- or D₂-like receptors.

4.5. Conclusions

A blockade of the D₂ receptor with haloperidol enhanced elbow flexion force tremor at 60% MVC but not 10% MVC. Haloperidol also decreased the voluntary activation of the unfatigued elbow flexors: an effect that was exacerbated in the presence of fatigue. These findings provide evidence that D₂ receptors relate to physiological tremor generation during sustained muscle contractions, particularly during efforts that require a substantial voluntary drive to the muscle. Our results also suggest that the D₂ receptor has a critical role in the

volitional drive to activate muscles during maximum effort contractions. In the presence of elbow flexor fatigue, a D₂ receptor blockade enhances the exercise induced decline in muscle force and voluntary activation, indicating that CNS dopamine pathways contribute to central fatigue. These effects can be attributed to changes in the activity of dopamine at supraspinal D₂ receptors, particularly within the nigrostriatal pathway.

Conflict of interest

There are no competing interests for the authors to declare.

Contributions

All experimental testing was performed within the School of Allied Health Sciences at Griffith University, Gold Coast Campus, Australia. All of the authors listed in this study qualify for authorship. J.R.T, J.J.K, M.G.T conceptualised and designed experiments. J.R.T collected data. J.R.T analysed data. J.R.T, J.J.K drafted manuscript. J.R.T, J.J.K, M.G.T approved final version of the manuscript.

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Legends to figures

Fig. 1. Experimental setup (A) and contraction protocol within both experiments (B). The participants sat upright and coupled at the wrist to an isometric torque transducer that positioned the elbow at 90 degrees of flexion. EMG signals were recorded from the biceps and triceps via bipolar surface electrodes placed over each respective muscle belly, and electrical stimulations were delivered via surface electrodes placed over the biceps. For *Experiment 1*, baseline maximum voluntary contractions (MVCs) were performed to determine each participant's peak maximum elbow flexion torque. The participants then performed 3 sustained submaximal isometric contractions at 10% and 60% MVC for 10 s in order to assess force tremor characteristics. For *Experiment 2*, voluntary activation characteristics were determined by performing brief maximum elbow flexions, where a single electrical pulse was applied to the elbow flexors at rest (resting twitch, open arrows) or during the MVC (superimposed twitch, closed arrows). The voluntary activation characteristics of the fatigued elbow flexors were then assessed proceeding 6 sustained maximum contractions, where superimposed twitches and resting twitches were once again obtained. Each of the 6 sustained maximum contractions continued until elbow flexion torque declined to below 60% MVC. With the exception of the fatigue protocol, adequate rest was given between all contractions within each experiment so that fatigue was tightly controlled.

Fig. 2. Representative force tremor data of a participant for the placebo (PLA) and haloperidol (HAL) conditions. Isometric elbow flexion force is presented for a 10% maximum voluntary contraction (MVC) trial following the administration of placebo (A) and haloperidol (B). Data is also presented for a 60% MVC following administration of placebo (C) and haloperidol (D). The lower panels contain the corresponding power spectrum for

10% MVC (E) and 60% MVC (F). Overall, participants exhibited greater force fluctuations during the isometric elbow flexions after haloperidol administration, particularly at 60% MVC. There were also distinct increases in power for the dominant tremor frequency (during 60% MVC) following haloperidol ingestion.

Fig. 3. The coefficient of variation (CV) (A) and the peak power amplitude in the 8-12 Hz bandwidth (B) for elbow flexion force at 10% and 60% maximum voluntary contraction (MVC). Data are presented for the placebo (PLA) and haloperidol (HAL) conditions. A higher CV suggests greater variability in isometric force regardless of contraction intensity. The filled circles represent the group means ($\pm$ group SD) and the grey lines represent CV or peak power data for each participant ($n = 10$). (*) indicates a significant difference at $p < 0.05$, (†) indicates $p < 0.01$, and (‡) indicates $p < 0.001$.

Fig. 4. The time to fatigue (A), area of elbow flexion torque (B) and area of biceps EMG (C) during the sustained maximum fatiguing elbow flexions for the placebo (PLA) and haloperidol (HAL) conditions. Six maximum contractions were performed until elbow flexor torque decreased to 60% MVC. Each contraction was separated by 90 s of rest. The data is presented as group means ($\pm$ group SD). (*) indicates a significant difference at $p < 0.05$, and (†) indicates $p < 0.01$.

Fig. 5. Rating of perceived elbow flexor fatigue changes for the six sustained MVCs within the placebo (PLA) and haloperidol (HAL) sessions. Each sustained contraction was separated by 90 s of rest, in which the measures for perceived elbow flexion fatigue were obtained via a CR10 Modified Borg Rating of Perceived Exertion (RPE) Scale. The data is presented as group means ($\pm$ group SD). (†) indicates a significant difference at $p < 0.01$.

Fig. 6. Elbow flexor superimposed twitch (A) and resting twitch (B) traces for a participant for the placebo and haloperidol conditions. Pre-fatigue trials are presented in the left panels and post-fatigue trials are presented in the right panels. Five superimposed and resting twitches are overlaid for each pre-fatigue and post-fatigue set. Twitches were elicited via a single supramaximal electrical stimulation of the elbow flexors. Arrows represent the onset of stimulation.

Fig. 7. Superimposed twitch torque (A), resting twitch torque (B), and level of voluntary activation (C) for the placebo (PLA) and haloperidol (HAL) conditions. The respective pre-fatigue and post-fatigue changes are shown. For panel (C), the level of voluntary activation (%) was determined via the equation: $(1 - \text{superimposed twitch}/\text{resting twitch}) \times 100$. The filled circles represent the group means ($\pm$ group SD) and the grey lines represent superimposed twitch, resting twitch or voluntary activation data for each participant ($n = 8$). (*) indicates a significant difference at $p < 0.05$, (†) $p < 0.01$, (§) $p < 0.001$.

A **B**