Investigating the modulation of methylphenidate's effects on impulsivity by fluoxetine

by

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Abstract

The co-prescribing of methylphenidate (MPH) and a selective serotonin reuptake inhibitor for patients presenting co-morbidly with both attention deficit/hyperactivity disorder and depression or anxiety is in some cases recommended. Little research has been conducted on the specific cognitive and behavioural outcomes of this. Studies with rats have shown that SSRI's potentiateMPH-induced dopamine release in the pre-frontal cortex, hippocampus and nucleus accumbens, as well as enhancing MPH-induced hyper-locomotion(Borycz, Zapata, Quiroz, Volkow, & Ferré, 2008; Weikop, Yoshitake, & Kehr, 2007b). Impulsivity is a behavioural construct with dissociable sub-types, of which one, 'action restraint', has been consistently shown to be associated with increased dopamine activity in the mesolimbic system, including the nucleus accumbens. It was hypothesised that rats would make more 'no-go' errors in a Go/No-Go task, indicative of an increase in 'action restraint' type impulsivity, when co-administered fluoxetine (FLX) and MPH compared to either drug administered alone. Although this was not shown in the current study, tentative evidence was found to suggest that the combination of these drugs may negatively impact on attention, based on a decrease in 'go' accuracy. A second subtype of impulsivity, "action cancellation", was tested using a new variant of the Stop-Signal Reaction Time (SSRT) task that we have developed for rats.Studies show that this subtype of impulsivity seems to be unaffected by changes in dopamine activity, but is improved by increases in norepinephrine. In the Weikof study mentioned above, the SSRI citalopram enhanced not only MPH-induced dopaminerelease, but also norepinephrine release in the nucleus accumbens. Thusit was hypothesised that FLX may potentiate MPH's impulsivity-reducing effects as measured by stopping latency in the SSRT. We were not able to show this in the current study, however the demonstration that lower doses of MPH reduced stopping latency, consistent with previous versions of the SSRT, validated the new version developed for the current study. A final experiment revealed a rapid, short-term increase in locomotor activity when rats were co-administered FLX and MPH, an effect not present when either drug was administered singly. This synergistic effect replicates previous findings, and indicates a potentiation of dopamine release in the nucleus accumbens, as was found in previous studies. Although FLX was not found to moderate MPH's effects on impulsivity in the current study, synergistic effects of the two drugs were effects were found on motor activity and potentially on attention also. This is an indication of the value of further research into specific behavioural and cognitive process that may be affected by co-administration of MPH and an SSRI.

Acknowledgements

Table of Contents

1. Investigating the modulation of methylphenidate's effects on impulsivity by fluoxetine

1.1. Attention-deficit/hyperactivity disorder (ADHD)

ADHD is the most common psychiatric disorder amongst children, with a word-wide prevalence of 5.9-7.1% (Willcutt, 2012). It occurs at around the same rate across all countries from which studies are available, including New Zealand (Polanczyk, Willcutt, Salum, Kieling, & Rohde, 2014). Few disorders have generated the amount of controversy and debate that surrounds ADHD, for several reasons. The largest demographic affected by this disorder is children, in which case the power lies with parents and teachers to recognise the problem, and decide whether to pursue a diagnosis and begin a medication regime, with the child themselves often having little input or understanding. Some believe that parents are choosing to medicate childhood behaviour that is not pathological, but simply at a further end of the continuum than what is considered the 'norm'. This is known as the social construct theory of ADHD (Timimi & Taylor, 2004). Adding to the controversy is the fact that the medications used to treat ADHD are psychostimulants that at higher doses can become addictive.

Although the disorder often becomes less of a problem as the child grows older, with symptoms sometimes diminishing by adulthood, around 30-60% of children with ADHD will still present with a clinical diagnosis as adults (Faraone & Beiderman, 2005). The DSM-V contains the same diagnostic criteria for both children and adults, however an adult diagnosis requires only five of the 18 symptoms, compared to the 6 that children must present with (American Psychiatric Association, 2013).

The DSM-V states that ADHD symptoms must be present to the point of "significant impairment in social, school, or work functioning." If an individual's symptoms are primarily from the 'inattentive' category, which includes 9 symptoms such as "difficulty sustaining attention", "has difficulty with organisation" and "is easily distracted", they will earn a diagnosis of ADHD with an inattentive presentation. If the symptoms are mainly from the 'hyperactive/impulsive' category, containing items such as "fidgeting", "extreme restlessness", "blurting out" answers inappropriately, and "difficulty waiting", they will be given a hyperactive/impulsive presentation (American Psychiatric Association, 2013, p.159) Patients with symptoms distributed across both categories are classed as having a combined presentation. As well as these fairly qualitative, observable behavioural indicators, patients

with ADHD tend to score below average in objective, computer-based cognitive-behavioural tests of attention and impulsivity, which is a critical argument against the social construct theory mentioned above. For the current study, the construct of impulsivity will be focused on, in the context of ADHD.

1.2. Impulsivity

Impulsivity is a naturally variable characteristic amongst individuals, with abnormally high levels of impulsivity being implicated in many other psychiatric disorders besides ADHD, such as schizophrenia and depression. Impulsivity can be broadly described as a tendency to act or make decisions quickly and without proper consideration of the consequences. In behavioural terms, it is the failure to adjust a pre-potent response to a stimulus when the adjustment will result in a better outcome. This broad definition can be broken down into various sub-categories of impulsive behaviour or cognition that can vary along different continuums within the same individual, and be differentially affected by procedural, pharmacological or neural manipulations. One of these subtypes of impulsivity is 'response inhibition'- the extent to which an individual has the ability to inhibit a pre-potent response that is no longer appropriate for the context. This is also described as 'motoric impulsivity'. This category can be even further dissociated into 'action restraint' and 'action cancellation' (Eagle, Bari, & Robbins, 2008). The former refers to the process of withholding a formerly or usually appropriate response due to a change in cue or context, signalling a switch in the reinforcement value of responding and not responding. The key distinction here is that the inhibition happens prior to the initiation of the response. A commonly used task to assess this ability in humans is the Go/No-Go (or the very similar variant the Continuous Performance Task), in which participants must make a response to a certain stimuli presented one at a time (e.g. letters or shapes), but withhold that response to other stimuli. This allows for dissociable measures of impulsivity (errors of commission, or making a 'go' response to 'no-go' stimuli) and attention (omissions, or not making a response to 'go' stimuli, *plus* errors of commission). Action cancellation on the other hand denotes the inhibition of an often reinforced response after that response has already been initiated, due to the appearance of a contextual cue that signals a reinforcement value in stopping that response. This can be tested using the Stop Signal Reaction Time task (SSRT), the set-up of which is very similar to the Go/No-Go task, however the timing of the 'no-go' (or in this case, 'stop') signal is

changed. Instead of appearing *instead* of the 'go' signal, it appears *after*, requiring the individual to inhibit the go response that they have already initiated. As is evident from its description, this task clearly measures the ability to cancel an already initiated action, distinct from the Go/No-Go task which taps into the ability to not initiate an action.

Both action restraint and cancellation are impaired in ADHD patients. Boonstra, Oosterlaan, Sergeant, and Buitelaar (2005) found in a meta-analysis of 13 studies comparing adults with ADHD to controls, a moderate effect size for more commission errors in continuous performance tests (CPT) in the ADHD group. The version of the CPT they examined was the multi-health system standard task, or Conners' CPT (Conners, 2000), a Go/No-Go task with high attention requirements. Chamberlain et al. (2011) conducted a meta-analysis across 13 studies investigating the performance of ADHD children and adults using the CANTAB, and found a significantly longer stop-signal reaction time in the ADHD group.

Until recently the two seemingly similar constructs of action restraint and cancellation were considered to be essentially the same process with different timing, and these tasks were used interchangeably to measure what was thought of as one overarching trait. They have now been consistently shown to recruit different neurochemical systems, and show impairment/improvement independently of one another (Eagle et al., 2008). For example, Robinson et al. (2009) found that high or low baseline levels of impulsivity in an action-restraint measuring task, the five-choice serial reaction time task (5-CSRTT) were not associated with impulsivity levels in the SSRT in rats. Throughout the rest of this introduction, these neurochemical dissociations between action restraint and cancellation will be explored further in the sections discussing the involvement of dopamine, norepinephrine, and serotonin in impulsivity.

1.3. Psychostimulant Treatment

The most commonly prescribed medications for ADHD in NZ are methylphenidate (MPH), better known by its brand name Ritalin, and dextroamphetamine, an enantiomer of amphetamine (AMPH) (Ministry of Health, 2001). Both of these drugs are psychostimulants, acting primarily on the dopamine (DA) and norepinephrine (NE) transporters as reuptake inhibitors, thus boosting activity of the DA and NE systems. There are some crucial

differences between them however. AMPH has an affinity for the serotonin re-uptake transporter (SERT), whereas MPH has almost no affinity for the SERT (Kuczenski & Segal, 1997). Additionally, amphetamine has a more complicated mechanism of action, not only blocking reuptake of the above mentioned neurotransmitters by inhibiting transporters, but also reversing transporter function, resulting in an efflux of the neurotransmitter (Robertson, Matthies, & Galli, 2009; Seiden, Sabol, & Ricaurte, 1993).

The majority of evidence for the efficacy of psychostimulant treatment in improving ADHD symptoms comes from subjective, observational rating scales completed by parents or teachers, or in adults, self-rating scales. These assess the level of behavioural and cognitive improvement in various everyday situations, closely resembling the DSM-V diagnostic criteria. Studies of this type have consistently found that MPH is effective in normalising the generally impulsive, hyperactive, and inattentive behaviour of children and adults with ADHD (Carlson, Pelham, Milich, & Dixon, 1992; Klein & Abikoff, 1997). Fewer studies have investigated the behavioural or cognitive effects of MPH using objective tests and tasks which indicate the level of functioning of specific neural processes. These types of studies are extremely useful, as they tell us not just that the drug works on a practical level, but also can illuminate how the drug is working, and help us to more exactly pinpoint the drug effects on specific symptoms, as opposed to overall clinical presentations which various aspects of the disorder may be contributing to. With regards to impulsivity, it has been shown that MPH reduces commission errors in the Go/No-Go or CPT, and reduces stopping latency in the SSRT of children and adults with and without ADHD (Aron, Dowson, Sahakian, & Robbins 2003; Broyd et al., 2005; DeVito et al., 2009; Groom et al., 2010; Nandam et al., 2011; Turner, Blackwell, Dowson, McLean, & Sahakian, 2005). A recent and thorough meta-analysis conducted by Coghill et al. (2014), which included 16 double-blind placebo-controlled crossover studies investigating the combined effect of acute (in all cases except one) MPH treatment on a roughly equal number of SSRT and Go/No-Go tasks, found that MPH significantly reduced impulsivity in children diagnosed with ADHD.

AMPH has consistently been shown to improve ADHD symptoms in studies using subjective ratings scales as assessment tools (e.g. Stein et al., 2011; and see Weyandt et al., 2014 for a review). However, studies assessing the effectiveness of AMPH using objective neuropsychological tests of impulsivity provide contrasting results (see section 5).

The efficacy (in general terms) of psychostimulants led to the formulation of the dopamine hypothesis of ADHD. This posits that dopamine activity is related to impulsivity and cognitive function in the form of an 'inverted U-shaped' curve. Individuals diagnosed with ADHD have too low a level of dopamine, resulting in impaired cognitive functioning, thus increasing it to a normal level improves cognition. Past a certain, optimal level of dopamine though, cognitive function declines (Swanson et al., 2007).

1.4. Dopamine and Impulsivity

Dopamine has been clearly shown to be implicated in impulsivity, although the relationship is complicated. Most of the research that will be discussed here is pre-clinical, using rats to investigate the behavioural effects of dopaminergic manipulations. This type of research has the advantages of being able to test thorough dose-response curves that utilise higher doses than would be ethical to test in humans, look at the effects of several drugs administered at once, directly measure neurotransmitter activity in the brain (either in vivo or post-mortem), and administer drugs directly to different areas of the brain. It also allows for a much greater degree of control over pre-experimental differences in an individual's environment and development than human research.

1.4.1. Dopamine and action restraint

Action restraint is consistently negatively affected by dopaminergic manipulations that increase DA transmission and levels in sub-cortical structures such as the striatum and nucleus accumbens (NAC). The task that is most often used for measuring action restraint in animals is the 5-choice serial reaction time task, analogous to the CPT. Rats are trained to poke their nose into whichever one of a series of 5 holes a light appears in, to obtain a food reward. The beginning of a trial is signalled by a light or a tone, followed by a delay before the light appears in one of the holes. The measure of impulsivity is the amount of trials in which the animal makes a premature response- that is, making a nose-poke into any of the holes during the delay. Premature responding (PR) is thought to reflect an inability to wait, to inhibit a pre-potent action until it is appropriate for the context (Robbins, 2002). GBR12909, a selective dopamine transporter (DAT) inhibitor, increases PR in the 5-CSRTT (Baarendse & Vanderschuren, 2012; Fernando et al., 2012; Wiskerke et al., 2011). Delving deeper into the exact dopaminergic mechanisms of this effect, Pezze, Dalley, and Robbins (2007)

demonstrated that the D1 receptor agonist SKF38393 infused into the NAC core before the task increased PR. Note that several earlier studies did not find an effect of the same agonist when it was administered systemically (Passetti, Dalley & Robbins, 2003a) or infused directly in the mPFC (Granon et al., 2000). Conversely, D1 antagonists reduce PR from baseline levels (Harrison, Everitt & Robbins, 1997; Pattij, Janssen, Vanderschuren, Schoffelmeer, & Van Gaalen, 2007; van Gaalen, Brueggeman, Bronius, Schoffelmeer, & Vanderschuren, 2006; Zeeb, Wong, & Winstanley, 2013). These effects were exerted on PR with no significant alteration in measures of general motor arousal, for example response latency, indicating that the interference is occurring at the level of response selection rather than a general lowering or enhancement of activity. Interestingly, a D2 and mixed D2/3 agonist reduced PR in rats selected for high impulsivity in the 5-CSRTT (Fernando et al., 2012). This is likely to be due to the activation of D2/3 autoreceptors that regulate neurotransmission from mesolimbic DA neurons extending to the NAC, with preferential activation of these leading to decreased DA activity.

The stimulant amphetamine (AMPH), a DAT blocker that induces a large rise in extracellular DA levels, has been demonstrated in numerous studies to dose-dependently increase PR in rats performing the 5-CSRTT (Baarendse & Vanderschuren, 2012; Paterson, Ricciardi, Wetzler & Hanania, 2011; Wiskerke et al., 2011). Murphy, Robinson, Theobald, Dalley, and Robbins (2008) found the dose-dependent AMPH-induced increase in perseverative responding in a 'forced choice' variant of the 5-CSRTT using only one available hole, which reduces the attentional demands of the task. Interestingly, Hayton, Maracle, and Olmstead (2012) found that while amphetamine increased PR in both short and long fixed inter-trial interval (ITI) conditions, there was no significant change in the variable ITI condition, leading them to suggest that amphetamine may disrupt an organism's ability to time intervals. Blokland, Sik, and Lieben (2005) found an AMPH-induced increase in PR in a task that required rats to hold a panel down until a tone was presented after a variable delay. These doses of amphetamine did not affect break point in a progressive ratio schedule of responding for reinforcement, ruling out the alternative explanation of increased motivation. AMPH's impact on action restraint seems to critically involve DA release in the NAC, as dopaminergic depletion in this region decreases AMPH-induced PR (Cole & Robbins, 1989).

While dopamine D1 receptor antagonists have been shown to influence baseline PR in the 5-CSRTT, this effect has generally not been shown when D2 antagonists have been administered in the same paradigm (e.g. Blokland et al., 2005; Harrison et al., 1997). D2

receptors however do appear to play a major role in action impulsivity produced by AMPH. A D2 antagonist injected into the core of the NAC attenuated the dose-dependent relationship between AMPH and PR (Pattij et al; 2007), this effect also being observed with a systemically administered D2 antagonist. (Van Gaalen, Unger, Jongen-Relo, Schoemaker, & Gross, 2009; Zeeb et al., 2013) The attenuating effect of D2 antagonists has been observed in other manipulations that increase both dopaminergic transmission and PR, such as the administration of cocaine (Van Gaalen et al., 2006) and lesions of the medial pre-frontal cortex (Pezze, Dalley & Robbins, 2009), suggesting that perhaps D1 receptors are more involved in maintaining tonic dopaminergic transmission while D2 receptors are responsible for the sharp rises in dopamine brought about by pharmacological agents or brain pathology.

MPH, like AMPH, is also a stimulant, with an affinity for the DA transporter as well as the NE transporter (NET). Unlike AMPH though, MPH has only a negligible affinity for the SERT. The other major difference in MPH's neurochemical profile is that it has a higher affinity for the NET than the DAT, whereas AMPH inhibits both relatively equally. Kuczenski and Segal (2001) tested rats' accumbal neurochemical response to acute doses of MPH and AMPH. They found that at the lowest dose tested (0.5 mg/kg i.p.), MPH did not induce a significant increase in DA levels in the NAC. This dose is considered to be 'clinically relevant', in that it produces a blood plasma level of MPH within the range of that which is observed in patients with ADHD after they have taken a prescribed oral dose that is effective in reducing their behavioural symptoms (between 8-40 ng/mL) (Berridge et al., 2006). However a clinically relevant dose of AMPH (0.25 mg/kg i.p.) produced a 250% rise in extracellular accumbal DA. This was significantly more than even the highest dose of MPH tested (2.5 mg/kg), which induced an increase of around 160% of baseline. In contrast to the DA response, the NE response to 0.5 mg/kg of MPH was significant, with NE levels rising to around 160% of baseline (as measured in the hippocampus). This was still lower than the NE rise in response to 0.25 mg/kg AMPH (around 225% of baseline, similar to the DA response), however the two higher doses of MPH (1.0 and 2.5 mg/kg) produced a much higher rise in hippocampal NE levels than the dose of AMPH (around 300% and 350%, respectively). Berridge & Devilbiss (2011) found using microdialysis that low, clinically relevant doses of MPH preferentially increase extracellular DA and NE in the prefrontal cortex, compared with other areas including subcortical structures.

MPH's flatter and overall lower dopamine dose-response curve compared to AMPH is reflected in its effect on action restraint in rats, which has almost exclusively been

measured using the 5-CSRTT. Unlike AMPH, MPH has inconsistent effects on rat's rate of PR in the 5-CSRTT. Low, clinically relevant doses have been shown to reduce PR when animals were not highly trained on the task (Puumala et al., 1996), or when PR was not punished using a time-out (Bizarro, Patel, Murtagh, & Stolerman, 2004). However most 5-CSRTT investigations using low doses have not found any significant effect of MPH (Paterson et al., 2011; Fernando et al., 2012; Navarra et al., 2008; Milstein, Dalley, & Robbins, 2010; Robinson, 2012). Higher doses have provided varying results as well. While Fernando et al. (2012) and Paterson et al. (2011) found that 2 mg/kg and 3 mg/kg of MPH did not affect PR, Milstein, Dalley and Robbins (2010) found a significant impulsivity-increasing effect of these moderate doses. High doses of 5 mg/kg and 10 mg/kg were found by Navarra et al. (2008) and Pattij, Schetters, Schoffelmeer and Van Gaalen (2012) to significantly increase PR, however Robinson (2012) found that neither low (1 mg/kg), moderate (3 mg/kg) nor high (10 mg/kg) oral doses of MPH affected PR. Also using oral administration, Pattij et al. (2012) actually found a strong trend for 1 mg/kg of MPH to decrease PR (p=0.055). This could have been due to oral administration of the drug, whereas most other studies have used intraperitoneal (i.p.) injection. Although they did not use the 5-CSRTT, Hill, Covarrubias, Terry and Sanabria (2012) found that MPH increased inhibitory capacity in an action-withholding task using a fixed minimum interval schedule of reinforcement, essentially measuring a very similar construct to the 5-CSRTT.

One may notice that with the exception of one, all of the animal experiments described above have used the 5-CSRTT to measure the effects of MPH on impulsivity. As discussed earlier, this is considered to be an analogue of the CPT used to detect motor impulsivity in humans. However, the CPT itself seems to be poorly characterised, with many different versions in use. Most, including the widely used Conners' CPT, are essentially a Go/No-Go task with higher attentional requirements, in which the participant in each trial must decide whether to make a response or not, based on the information they are presented. This is slightly different to the 5-CSRTT in which the animal must make the learned response on every trial, holding it 'on line' until the appropriate moment. Other versions of the CPT are closer in their requirements to the 5-CSRTT, with participants reading a sequence of letters, making a response if the sequence matches a target sequence (Mathias, Marsh, & Dougherty, 2002). Trials vary in the amount of the sequence that matches the target- the most difficult trials will differ from the target on the last letter only. Participants make premature responses when they make a response before they have finished reading the sequence. This

version of the task manages to capture the same 'waiting' aspect of action restraint as the 5-CSRTT, however also has a Go/No-Go aspect to it as a response is only required on some trials. Though Go/No-Go type tasks and 'waiting' tasks both provide a measure of motor impulsivity, the slightly different cognitive and behavioural requirements may recruit some dissociable neural processes and thus be affected differently by pharmacological manipulations, thus accounting for the much more consistent impulsivity-reducing effect of MPH in humans, in contrast with rodent studies, most of which fail to show an effect of MPH.

1.4.2. Dopamine and action cancellation

One of the major distinctions between action restraint and cancellation is that DA does not seem to play a critical role in action cancellation. Although MPH does improve SSRT performance in both humans (see section 3), and rats (Eagle, Tufft, Goodchild, & Robbins, 2007) more specific dopaminergic manipulations have not been shown to affect this construct. Lesions of the NAC core do not alter rats' baseline SSRT (Eagle & Robbins, 2003a). Neither do mixed D1/D2 antagonists, nor do they affect an MPH-induced improvement in inhibition (Bari et al., 2011; Eagle et al., 2007). Effects were also failed to be obtained with D3 and D4 agonists and antagonists (Bari & Robbins, 2013). Likewise, DA reuptake inhibitors such as GBR 12909 do not affect stopping speed in the SSRT (Bari, Eagle, Mar, Robinson, & Robbins, 2009). In the human research, the DA precursor L-DOPA, which increases DA synthesis and release, does not affect the baseline SSRT of children with ADHD (Overtoom et al., 2003).

As was illustrated in the previous section, MPH induces a similar efflux of NE but a much higher efflux of DA at a comparable low dose to MPH, and consistently increases rats' impulsivity in measures of action restraint such as the go/no-go and 5-CSRTT. With regards to humans, AMPH, unlike MPH, fails to ameliorate children with ADHD's Go/No-Go commission errors compared with placebo (Wilson, Cox, Merkel, Moore, & Coghill, 2006), and also fails to have any effect on commission errors in volunteers without ADHD (Sofuoglu, Mooney, Kosten, Waters, & Hashimoto, 2011). When the difficulty of the Go/No-Go task is increased by mis-cueing, amphetamine actually increases impulsivity, in line with the animal research (Fillmore, Rush, & Marczinski, 2003). Contrastingly, amphetamine has been shown to improve action cancellation in the SSRT in healthy individuals (de Wit, Enggasser, & Richards, 2002), and also rats (Eagle & Robbins, 2003b, Eagle et al., 2009;

Feola, de Wit, & Richards, 2000), the higher DA efflux not counteracting the effect of the NE as it does in the Go/No-Go.

The apparent lack of involvement of DA in action cancellation has led some researchers to postulate that the decrease in SSRT seen with stimulants is most likely due to their NE-increasing properties.

1.5. Norepinephrine and impulsivity

NE appears to be a critical neurochemical component for both action restraint and cancellation. Most of the support for this comes from the research surrounding one of the newer ADHD treatments, atomoxetine (ATX). This drug has a selective affinity for the NET only, increasing levels of NE and DA in the prefrontal cortex (as NET in the PFC contributes to the re-uptake of both NE and DA), but unlike MPH, does not increase DA in the NAC or striatum at therapeutic doses (Bymaster et al., 2002). ATX has proven its efficacy in child and adult ADHD patients, both using observational behavioural ratings scales (e.g. Durell, Adler, Wilens, Paczkowski, & Schuh, 2010; Kratochvil et al., 2002; Schwartz & Correll, 2014), and using specific impulsivity tasks, including the SSRT (Chamberlain et al., 2006, 2007) and go/no-go (Shang & Gau, 2012; Wehmeier et al., 2011). Schulz et al. (2012) compared MPH and ATX in their effect on go/no-go performance and found that both produced a similar, significant reduction in commission errors. Animal models of impulsivity have also consistently demonstrated significant, dose-dependent improvement with ATX in the 5-CSRTT and SSRT (Baarendse & Vanderschuren, 2012; Fernando et al., 2012; Navarra et al., 2008; Paterson et al., 2011; Robinson et al., 2008b; Robinson, 2012). Desipipramine, another NET inhibitor, has been shown to decrease stopping latencies in humans (Overtoom et al., 2003) and 5-CSRTT commission errors in rats (Pattij et al., 2012). The clinical use of NET inhibitors is rising, as around 35% of people with ADHD do not respond to stimulant treatment, showing little improvement in their symptoms (Hodgkins, Shaw, Coghill, & Hechtman 2012). Thus NET inhibitors have become the 'second call' for these patients.

In section 1.3 the inverted-U theory of dopamine in ADHD was mentioned. This came about mainly due to the observation that stimulants improved ADHD symptoms. However when looking specifically at impulsivity, a slightly different picture emerges. Berridge & Devilbiss (2011) observed that low doses of MPH act mainly on the NET in

frontal areas. These low doses of MPH seem to be the most effective at improving measures of impulsivity- not only has the animal literature reviewed above shown this, but also human studies. Action restraint as measured by commission errors in the CPT was decreased with a lower, but unaffected by a higher dose of MPH (O'Toole, Abramowitz, Morris, & Dulcan, 1997), and action cancellation as measured by SSRT performance was less improved with higher MPH doses than lower doses, (Konrad, Günther, Heinzel-Gutenbrunner, & Herpertz-Dahlmann, 2005; Tannock, Ickowicz, & Schachar, 1995).

From this it could be suggested that stimulants improve symptoms of impulsivity in people with ADHD not for their effects on the dopaminergic system, but their norepinephrine-increasing properties. In the case of action restraint, the rise of dopamine beyond that induced by a low dose of MPH appears to actually increase impulsivity, whereas action cancellation appears to be unaffected by specific dopaminergic manipulations. The different effects of AMPH and MPH fit well into this theory. As AMPH induces a similar efflux of NE but a much higher efflux of DA at a comparable low dose to MPH, (Kuczenski & Segal, 2001) this could explain why even at low doses, AMPH tends to increase measures of action restraint.

1.6. Serotonin and impulsivity

1.6.1. Serotonin and action restraint

Serotonin (5-HT) has long been known to be implicated in inhibitory processes. Post-mortem examinations have demonstrated that victims of suicide (an act associated with high levels of impulsivity, as evidenced by behavioural impulsivity measures of suicide attempters) have lower levels of 5-HT and SERT than depressed controls who have died by other causes (Kamali, Oguendo, & Mann, 2001). A more reliable method of investigating the relationship between 5-HT and impulsivity in humans is the manipulation of 5-HT levels via acute tryptophan depletion (ATD) - tryptophan being the amino-acid precursor for 5-HT. Several studies have shown that ATD impairs action restraint in humans, as measured by the CPT (Dougherty et al., 2007; Walderhaug et al., 2002, 2007).

Findings from animal research have been in agreement with the human data. Methods of global 5-HT depletion used on animals are much more effective than those used in

humans, with the most common being 5, 7-DHT lesioning. This method decreases forebrain 5-HT levels to around 10% of baseline (Winstanley, Dalley, Theobald, & Robbins 2003b) allowing for strong impulsivity-increasing effects to be seen in the 5-CSRTT (Harrison et al., 1997, Carli & Samanin, 2000), 1-CSRTT (Winstanley et al., 2003b) and Go/No-Go (Harrison, Everitt, & Robbins, 1999).

In line with this, the increasing of global 5-HT levels through the administration of selective serotonin re-uptake inhibitors (SSRI's) such as citalopram, fluoxetine and paroxetine has been shown to decrease PR in the 5-CSRTT (Baarendse & Vanderschuren, 2012, Humpston, Wood & Robinson, 2013) as well as reduce response rate and increase reinforcement rate in the DRL-72 paradigm during which rats must withhold a response for 72 seconds (Marek, Li, & Seiden, 1989; Sokolowski & Seiden, 1999). However, in humans the acute administration of SSRI's has so far not produced any effects on impulsivity scores as measured by the CPT or Go/No-Go (Almeida, Glahn, Argyropoulos, & Frangou, 2010; Del-Ben et al., 2005; Iwamoto et al., 2008).

A further source of variation in serotonin functioning and levels is genetics, with data from this area providing yet more evidence for the reverse correlation between serotonin and impulsivity. Humans possessing the G allele of the C(−1019)G functional polymorphism of the HTR1A gene have lower serotonergic neurotransmission due to an over-expression of the 5-HT1A autoreceptor. The G allele has been associated with higher motor impulsivity as assessed by a subscale of the Barratt Impulsiveness Scale (Benko et al., 2010). Rats that have been genetically engineered to lack the SERT, which results in extracellular 5-HT levels that are nine times higher than those of wild-type rats (Kalueff, Olivier, Nonkes, & Homberg, 2010) show significantly less PR in the 5-CSRTT (Homberg et al., 2007).

The serotonin system is highly complex, with 14 known receptor family subtypes. The use of specific 5-HT receptor agonists and antagonists has revealed a picture that is much more complicated than a simple inverse correlation between 5-HT levels and impulsivity, of the type that has been observed with global 5-HT depletion. The receptors that have received the most attention in the area of impulsivity are the 2A and 2C, and clear dissociations have been demonstrated between these in terms of their moderation of action restraint. Winstanley, Theobald, Dalley, Glennon, and Robbins (2004) found that a 2A antagonist decreased PR in the 5-CSRTT, a seemingly paradoxical effect when compared with more general serotonergic manipulations. However, a 2C antagonist increased this measure of impulsivity, mimicking

the effect of global 5-HT depletion (see above). This implies that the 2C receptor plays a key role in serotonergic regulation of behavioural inhibition, and that the increases in impulsivity seen with global 5-HT depletion could be due to reduced activation of 5-HT2C receptors. Furthermore, in rats lesioned with 5, 7-DHT, which initiated the expected increase in impulsivity, the effect of the 2A antagonist was attenuated. The authors suggest that this may be because the impulsivity-reducing effect of 2A receptor antagonism is due to an unmasking of the effects of 5-HT at other receptors, which would be very much reduced in the lesion condition.

      5-CSRTT data from a number of other studies has produced a consistent pattern, with specific 2A antagonists reducing (Fletcher, Tampakeras, Sinyard, & Higgins, 2007) and 2C antagonists increasing PR (Carli, Baviera, Invernizzi, & Balducci, 2006; Fletcher et al., 2007; Higgins, Enderlin, Haman, & Fletcher, 2003; Paterson, Wetzler, Hackett, & Hanania, 2012; Winstanley et al., 2003a;). Ketanserin, an antagonist for both 2A and 2C, but with a far greater affinity for 2A (Barnes & Sharp, 1999), also decreases PR (Passetti et al., 2003a; Paterson et al., 2012; Talpos, Wilkinson, & Robbins, 2006). Conversely, 2C agonists decrease PR (Fletcher et al., 2007; Navarra et al., 2008). DOI, an agonist at both receptors, increases PR in both the 5-CSRTT (Koskinen et al., 2000) and a task which required rats to hold down a panel until a specific tone sounded (Blokland et al., 2005). The complete blocking of this effect of DOI by ketanserin, and the lack influence of the 2A/C antagonist SER082, which has a 10-fold higher affinity for the 2C than the 2A, shows that this impulsivity-increasing effect of DOI is likely due to stimulation of 2A receptors (Koskinen et al, 2000). Manipulations of 2A and 2C receptor functioning can not only affect baseline impulsivity, but also impulsivity induced by other substances. A 2C agonist (Fletcher, Rizos, Noble, & Higgins, 2011), and 2A antagonist (Carli et al., 2006; Higgins et al., 2003; Mirjana, Baviera, Invernizzi, & Balducci, 2004) reverse the increase in premature responses induced by the NMDA receptor antagonists dizocilpine and CPP.

      Although the bulk of the research has focussed on the 5-HT 2A and 2C receptors, there is evidence to suggest the 5-HT1A receptor also possesses a degree of influence over action restraint. The 1A agonist 8-OH-DPAT increased PR on the 5-CSRTT, with this effect attenuated by both systemic and dorsal raphe nuclei-specific pre-treatment with the 1A antagonist WAY-100635. (Carli & Samanin, 2000, Carli et al., 2006). Winstanley et al. (2003a), found that 8-OH-DPAT increased PR when administered systemically, but not when infused into the medial pre-frontal cortex (mPFC). This could be due to differential effects of

presynaptic 5-HT1A autoreceptors, which reduce 5-HT transmission when activated, and postsynaptic 1A receptors which have a range of effects on the postsynaptic neuron (see section 1.7.1).

1.6.2. Serotonin and action cancellation

Interestingly, researchers fail to find any effect of serotonergic manipulations on action cancellation, as measured by the SSRT, unlike the clear and consistent effects seen in measures of action restraint. For instance, when SSRT was measured in humans after ATD, no effect of the treatment was found (Clark et al., 2005; Crean, Richards, & de Wit, 2002), nor have researchers been able to find any effect of SSRI's on SSRT in humans (Chamberlain et al., 2006; Drueke et al., 2010; Nandam et al, 2011) or rats (Bari et al., 2009). 5, 7-DHT lesioning has also failed to produce any significant results in rats performing the SSRT (Eagle et al., 2009). It appears that the two types of impulsivity being examined are dissociated in terms of serotonergic involvement.

1.7. The influence of serotonin on dopamine activity

There is ample evidence to suggest that the 5-HT system exerts its influence on action restraint through complex upstream actions with the dopaminergic system. Before we examine this possibility however, we will first investigate the evidence supporting a modulatory role of the 5-HT system, at the receptor level, over DA activity in general. It is useful to break the interactions between the 5-HT and DA systems down to the receptor level, because as mentioned above, the 5-HT system is incredibly complex, and different receptors exert different effects on DA functioning.

1.7.1. The 5-HT1A receptor

The 5-HT1A receptor exists in two forms- as an autoreceptor located on the dendrites and cell bodies of 5-HT neurons in the raphe nuclei, and as a postsynaptic receptor located on the terminals of other neurons that receive input from 5-HT. The latter type, when activated, leads to inhibition of the post-synaptic cell's activity, and the former, through negative feedback, limits the firing of the 5-HT neuron and reduces levels of 5-HT in the synapse (Altieri, Garcia-Garcia, Leonardo, & Andrews, 2012). 5-HT innervation extending from the raphe nuclei is thought to provide net tonic inhibition (through 5-HT 2A and 2C receptors, which will be discussed below) over structures within the nigrostriatal system such as the

substantia nigra (SN) and ventral tegmental area (VTA), limiting the activity of dopaminergic neurons and, consequently, extracellular DA levels in the striatum (Haleem, 2015; Kelland, Freeman, & Chiodo, 1990). It is clear then that the 5-HT1A receptor must play a role in the complex interactions between 5-HT and DA.

Studies utilising the 5-HT1A full agonist, 8-OH-DPAT, demonstrated that systemic activation of 5-HT1A receptors abolishes dorsal raphe nuclei (DRN) 5-HT neuronal firing. Conversely, it increased both firing rate, and burst-pattern firing, of DA neurons in the VTA and SN at lower doses, whereas at higher doses, dopaminergic activity decreased in these regions (Arborelius et al., 1993a; Lejeune & Millan, 2000). This is in line with research that suggests that lower doses of 8-OH-DPAT preferentially activate the 5-HT1A autoreceptors, and it is not until the higher doses are reached that 5-HT1A post-synaptic receptors are occupied (Sharp, Bramwell, Hjorth, & Grahame-Smith, 1989; Yocca, Iben, & Meller, 1992). Activation of the autoreceptors would reduce raphe 5-HT output and disinhibit the DA circuits within the basal ganglia. Following studies showed that alongside this increased burst firing induced by 8-OH-DPAT was an increase in extracellular DA in the prefrontal cortex, but this was not observed in the basal ganglia structures themselves, such as the striatum or NAC (Arborelius, Nomikos, Hacksell, & Svensson 1993b; Rollema, Lu, Schmidt, Sprouse, & Zorn, 2000; Tanda, Carboni, Frau, & Di Chiara, 1994). In some cases, a high dose of 8-OH-DPAT actually reduced DA levels in the basal ganglia (Ichikawa & Meltzer, 2000). This is puzzling considering the increased activity of VTA neurons, which project to the NAC, and when activated increase DA levels in this structure.

Buspirone and ipsapirone are full agonists at 5-HT1A autoreceptors and partial agonists at post-synaptic receptors, and at lower doses preferentially activate the autoreceptors located on the bodies of serotonergic neurons (Celada, Bortolozzi, & Artigas, 2013). The systemic effects of these drugs on DA levels are slightly different to those of 8-OH-DPAT, increasing extracellular dopamine levels in the NAC and striatum as well as the PFC (Tanda et al., 1994, Ichikawa & Meltzer, 1999).

A major line of research contributing to knowledge of the 5-HT1A receptor's influence over DA activity is that regarding the extrapyramidal symptoms (EPS) that occur with classic antipsychotics, resulting from antagonism of the D2 receptor and consequent reduced DA transmission. (Glazer, 1999). Newer, atypical antipsychotics, which possess agonist capabilities at the 5-HT1A receptor, do not tend to give rise to these side-effects, and

also tend to improve the negative symptoms of schizophrenia. These features may be due to the increased DA levels in the PFC and striatum observed after administration of atypical antipsychotics, with higher doses inducing larger cortical increases than striatal, and the effect in the PFC being reversed by the 1A antagonist WAY-100635 (Rollema et al., 2000).

8-OH-DPAT blocks the EPS evoked by the antipsychotic haloperidol, or by monoamine depletion, both when it is administered systemically, or directly to the motor cortex or striatum (Mignon & Wolf, 2002; Shimizu, Tatara, Imaki, & Ohno, 2010), with this effect being reversed by WAY-100635. This effect still occurred after inactivation of 5-HT neurons, supporting the implication of post-synaptic 5-HT1A receptors on dopaminergic or other types of neurons (Mignon & Wolf, 2002). This is not to say that autoreceptors in the raphe nuclei do not play a part, as local application of 8-OH-DPAT to the medial raphe nuclei also blocked the EPS induced by the D2 antagonist raclopride (Wadenberg & Hillegaart, 1995).

In summary, it appears that activation of the 5-HT1A autoreceptor increases activity and dopamine levels in dopaminergic structures such as the VTA, NAC, SN and striatum, whereas activation at the postsynaptic form of the receptor increases cortical DA efflux. Activation at both receptor locations seems to contribute to the relief of EPS associated with reduced nigrostriatal dopaminergic function.

1.7.2. The 5-HT2A and 2C receptors

The 5-HT2A and 2C receptors seem to have opposing effects over DA activity. The dopaminergic behavioural effects of cocaine, including hyper-locomotion, self-administration, and conditioned place preference can be potentiated by various selective 5-HT 2C antagonists (Capriles, Watson, & Akil, 2012; Cunningham et al, 2011; Filip, Bubar, & Cunningham, 2004, Fletcher, Sinyard, & Higgins, 2006) while 2C agonists attenuate these dopamine-related behaviours (Filip et al., 2004; Fletcher, 2008; Grottick, Fletcher, & Higgins, 2000) as do 2A antagonists (Burton, Rizos, Diwan, Nobrega, & Fletcher, 2013)

In vivo measurements provide more direct evidence for the modulatory role of 2A and 2C receptors over dopamine neuron activity in both the basal and activated states. 2C antagonists and agonists, administered systemically, respectively increase and decrease the basal firing rate of dopamine neurons and dopamine outflow in areas including the striatum, NAC and PFC (de Deurwaerdere & Spampinato, 1999; Di Giovanni et al., 1999; Gobert et

al., 2000). 2C ligands show this same pattern in their influence on phasic, or active dopamine transmission, for example in conditions in which drugs that activate the dopamine system have been administered (Navailles, de Deurwaerdere, Porras, & Spampinato 2004; Pierucci, di Matteo, & Esposito, 2004; Porras, Matteo, de Deurwaerdere, Esposito, & Spampinato, 2002).

Basal dopamine activity does not seem to be affected by 2A antagonism (Berg, Harvey, Spampinato, & Clarke, 2008), leading many researchers to conclude that the 2A receptor does not play a role in this state. However 2A stimulation via an agonist increased basal dopamine release in the NAC, and this effect was attenuated by co-administration of 2A antagonist (Yan, 2000). A much clearer effect of 2A functioning can be seen in conditions of increased DA activity, with 2A manipulation producing the opposite set of results as 2C manipulations. When dopamine-activating drugs are administered, 2A antagonists inhibit the release of DA in the NAC and striatum (Liegeois, Ichikawa, & Meltzer, 2002; Lucas, de Deurwaerdere, Caccia, & Spampinato, 2000; Porras et al., 2002). Similarly, increased DA release in the NAC due to electrical stimulation of the DRN was attenuated by 2A antagonist (de Deurwaerdere & Spampinato, 1999). The mixed agonist DOI potentiates amphetamine-induced DA release in both the NAC and mPFC, with the effect being blocked by a selective 2A antagonist (Ichikawa & Meltzer, 1995; Kuroki, Meltzer, & Ichikawa, 2003).

In summary, the available literature strongly suggests that 5-HT2C receptors provide inhibitory control over both basal and activated accumbal DA activity, and that 5-HT2A receptors facilitate accumbal DA outflow in conditions of increased DA activation, but probably do not participate in basal DA regulation.

1.7.3. The 5-HT1B receptor

The 5-HT1B receptor, similarly to the 1A, acts as a presynaptic autoreceptor on 5-HT neurons (Adell, Celada, & Artigas, 2001) as well as being a heteroreceptor on the presynaptic terminals of other cells such as GABAergic neurons, and thirdly as a postsynaptic receptor (Sari, 2004).

Much of the evidence for 1B involvement in DA circuitry comes from its ability to modulate behavioural responses to cocaine by enhancing the cocaine-induced increase in dopamine outflow in the NAC (O'Dell & Parsons, 2004; Parsons, Koob, & Weiss, 1999). As well as blocking DA reuptake, cocaine blocks the SERT, and it seems that increased

activation of the 1B receptor plays a major role in the reinforcing effect of cocaine. Self-administration dose-effect curves of both the DA reuptake inhibitor GBR-12909 and cocaine are shifted to the left by various 5-HT1B agonists (Parsons, Weiss, & Koob, 1996, 1998), signifying more reinforcement is being gained from lower doses of these DA-releasing drugs. Pre-treatment with 1B agonists, as well as overexpression of 5-HT1B in the terminals of neurons projecting from the NAC to the VTA also increases the breakpoint of rats self-administering cocaine on a progressive ratio schedule, another signifier of an increased rewarding effect of the drug (Parsons et al., 1998, Pentkowski et al., 2012). Conversely, self-administration of cocaine microinjections to the VTA were extinguished by a 1B receptor antagonist, an effect which mimicked that of pre-treatment with a D1 antagonist (David, Segu, Buhot, Ichave, & Cazala, 2004). The locomotor effects of cocaine are also affected by manipulation of the 5-HT1B receptor, with overexpression of 1B receptors in the accumbens projections to the VTA enhancing the spontaneous locomotor response to a single dose of cocaine (Neumaier, Vincow, Arvanitogiannis, Wise, & Carlezon, 2002). Furthermore, a 1B agonist potentiated, and an antagonist diminished sensitisation of the locomotor response to cocaine after 5 consecutive days of co-treatment (Przegalinski, Papla, Siwanowicz, & Filip, 2004). The mechanisms behind these effects seem to involve indirect stimulation of DA neurons via GABAergic and/or glutamatergic pathways influenced by 5-HT1B receptors within the VTA. Alongside potentiating the cocaine-induced rise in accumbal extracellular DA, 1B activation enhances cocaine's diminishing effect on GABA levels in the VTA, as well as increasing accumbal DA and reducing GABA levels when administered alone (O'Dell & Parsons, 2004; Parsons et al., 1999). The fact that in the O'Dell and Parsons study the 1B agonist was administered locally to the VTA further supports this proposed mechanism. GABAergic neurons in the VTA exert tonic inhibitory control over NAC dopaminergic neurons. These GABA producing neurons possess 5-HT 1B heteroreceptors at their terminal regions, which when activated, decrease that neuron's activity, thus disinhibiting DA efflux in the NAC (Cameron & Williams, 1994; Johnson, Mercuri, & North, 1992). As 1B activation alone does not induce a rise in DA levels, it is likely that the DAT normally takes up the excess dopamine in the synapse, however the concurrent inhibition of the DAT caused by cocaine prevents this from happening.

The release of DA in the NAC is also tonically inhibited by glutamate via GABA, with glutamatergic projections from the hippocampus activating NMDA receptors on GABAergic cells in the NAC, which in turn inhibit dopamine firing (Charara & Grace, 2003;

Pitman, Puil, & Borglund, 2014; Sesack & Pickel, 1990). This mechanism may be responsible for the accumbal DA-increasing effects of 1B agonists microinjected into the hippocampus (Boulenguez et al., 1996, 1998), with 1B activation reducing the activity of the glutamatergic neurons extending to the NAC. This is supported by in-vitro studies demonstrating that 5-HT1B agonists applied to NAC slices decreases glutamatergic activation of medium spiny neurons (Muramatsu, Lapiz, Tanaka, & Grenhoff, 1998), as well as the demonstration of long term depression of glutamatergic transmission in the NAC by a locally administered 1B agonist (Huang, Yeh, Wu, & Hsu, 2013).

The ability of 1B receptors in the hippocampus to affect accumbal DA levels is not just restricted to local administration, with severance of the hippocampus-NAC connection abolishing the rise in extracellular DA levels brought about by systemic 1B agonist administration (Boulenguez et al., 1996), implicating the hippocampal glutamate projection to the NAC in the cocaine-sensitising effects described above.

In summary, it appears as though the 5-HT 1B receptor's permissive influence on DA activity in the NAC originates in both the VTA via GABA, and via glutamatergic mechanisms projecting from the hippocampus, as well as there being an influence on the nigrostriatal pathway via 1B receptors within the striatum.

1.8. The influence of serotonin on dopaminergic effects on action restraint

From the review above it is clear that the manipulations that tend to increase DA transmission or DA related behaviours also tend to increase action-restraint type impulsivity, and in fact the 5-HT receptors discussed above may exert their dissociable effects on action restraint through the different ways in which they moderate dopamine activity.

The modulatory effects of 5-HT 2A and 2C ligands on action restraint have been shown to be specific to the NAC by experiments using micro-infusions (Robinson et al., 2008a), with the infusion of other locations such as the pre- and infra-limbic cortices failing to produce any effects on baseline action restraint. This in itself implies that these effects are happening via an interaction with the DA system. Anastasio et al. (2011) found that the 5-HT2A antagonist M100907's PR-reducing effect on the DRL and 1-CSRT tasks was likely to be through an interaction with the DA system, as cocaine-induced impulsivity, which occurs via an increase in DA levels, was also attenuated by the 2A antagonist in these tasks. This

data is in line with previous research demonstrating an excitatory or permissive role of the 5-HT2A receptor in DA efflux, but goes further to suggest that this interaction can modify impulsivity as a behavioural outcome. Cocaine and amphetamine-induced PR in the 5-CSRTT has been reduced by both a 2C agonist and a 2A antagonist (Fletcher et al., 2011), as well as global 5-HT reduction via 5, 7 DHT lesions (Harrison et al., 1997). Koskinen and Sirvio (2001) investigated the 5-HT/DA interaction from the inverse perspective, and found that the impulsivity-increasing effect of 5-HT2A agonist DOI, was completely blocked by the systemic administration of a D1 antagonist, and attenuated by a D2 antagonist, at doses that did not affect PR themselves. Surprisingly, when DOI was injected into the NAC core and shell, no effect was observed. Along the same lines, Harrison et al. (1997) found that the increase in impulsivity produced by central serotonin depletion can be blocked by the D1 receptor antagonist SCH 23390.

These studies strongly suggest that both the general and receptor-specific serotonergic effects on action restraint that have been observed by many researchers may arise through a dopamine-serotonin interaction. This drives us to ask the question- can acute changes in the serotonin system caused by SSRI's moderate the outcomes of stimulants such as MPH on measures of impulsivity?

1.9. Selective Serotonin Reuptake Inhibitors and Methylphenidate

It was mentioned earlier in this introduction that a substantial number of children diagnosed with ADHD go on to suffer from the condition as adults, and in fact an Australian study found that adults aged 20-24 are the largest and fastest growing demographic to be diagnosed with ADHD and prescribed MPH (Karanges, Stephenson, & McGregor, 2014). Co-morbidity of ADHD and major depression (MD) or anxiety disorders rises with age, and a large-scale clinical study found that 35.71% of men, and 54.02% of women with ADHD also had MD (Turgay & Ansari, 2006). A report by Kessler et al. (2006) found that in the North American population they studied, adults with ADHD were almost 2.5 times more likely to experience MD and/or an anxiety disorder than the rest of the adult population. The most common pharmacological treatments for depression are selective serotonin reuptake inhibitors (SSRI's) such as fluoxetine or citalopram. These drugs, through their action at the serotonin transporter, cause synaptic levels of serotonin to rise and trigger a range of changes in the brain which begin to alleviate the symptoms of depression and anxiety after

approximately two to three weeks of administration (Blier & de Montigny, 1999). The American Academy of Adolescent and Child Psychiatry recommends the prescribing of both SSRI's and psychostimulants to patients with a comorbid ADHD/MD presentation, if one drug alone does not alleviate both sets of symptoms (Greenhill, Pliszka, & Dulcun, 2002).

The reviewed literature clearly shows that the serotonin system interacts with and influences the dopamine system. This leads to the question- how might the co-administration of SSRI's and MPH affect behaviour, specifically impulsivity? So far we have focussed on 5-HT/DA interactions at the level of individual 5-HT receptors. But SSRI's have a very broad effect on the 5-HT system and activate multiple receptors at once due to the rise in extracellular 5-HT in the synapse. While it is a fair prediction that an SSRI co administered with MPH may influence MPH's effects on impulsivity, to predict the nature of the effect it is necessary to look at studies that have examined the effect of SSRI's on dopamine release and dopamine –related behaviour induced by MPH and other dopaminergic drugs.

Neurochemical investigations using in-vivo microdialysis have found some interesting results in this regard. Weikop, Yoshitaki, & Kehr (2007b) gave rats a 2.5 mg/kg dose of MPH after the administration of the SSRI citalopram, which by itself does not affect DA levels, but significantly raises extracellular 5-HT levels. They found that the DA efflux induced by MPH in this condition was significantly higher than in the control condition, when it was preceded by a prior dose of MPH. This effect was observed in the NAC (in which MPH-induced NE was also observed to be potentiated), the hippocampus, and the prefrontal cortex. A second experiment investigated the PFC effects only (Weikop, Kehr, & Scheel-Kruger, 2007a), replicating them and further demonstrating that they could be completely blocked by pre-treatment with the 5-HT1A antagonist WAY-100635, suggesting that this receptor subtype is a key player in the synergistic effects of the two drugs- at least in the PFC.

Building on this purely neurochemical data, Borycz, Zapata, Quiroz, Volkow, & Ferre (2008) found that MPH-induced locomotion was increased in rats when MPH administration was preceded by the SSRI fluoxetine (FLX), while FLX alone did not have any effect on locomotion. This behaviour strongly suggests increased dopamine activity, however the authors did not directly measure DA levels. The authors also investigated MPH-induced locomotor activity after a pre-administration of the drug methamphetamine, which has an equal affinity for the SERT and the DAT, and significantly increases levels of extracellular 5-

HT. They found that compared with a MPH-MPH control condition, the pre-dose of methamphetamine increased MPH-induced locomotor activity. In this case they did measure DA levels in the nucleus accumbens and found that they were comparable to that of the group that received two MPH doses, however the dose of MPH used (10 mg/kg) was much higher than in the Weikop experiments, possibly introducing a ceiling effect. Additionally, in this experiment DA levels were only measured in the NAC shell, whereas Weikop et al. (2007b) did not specify but could have measured core levels. When the functioning of different 5-HT receptors was manipulated, it was found that the locomotor effects of preceding MPH with FLX or methamphetamine were fully attenuated by the pre-administration of the 5-HT1B antagonist GR55562, whereas the general 5-HT2 receptor antagonist ritanserin failed to. In line with this, the 5-HT1B agonist CP94253 dose-dependently potentiated the locomotor increase induced by MPH alone.

These three studies clearly demonstrate a moderating effect of SSRI's on the neurochemical and behavioural effects of MPH, and implicate the 5-HT 1A and 1B receptors in this process, at least in rats. But due to a lack of research on MPH specifically, it is necessary to examine the literature for more evidence concerning the effects of SSRI's on other DA-releasing agents. Both citalopram and FLX increased haloperidol-induced DA outflow in the rat striatum (Lucas et al., 2000). Huang, Ichikawa, Li, Dai and Meltzer (2006) found that the ability of risperidone to increase DA levels in the mPFC of rats was significantly augmented by the co-administration of citalopram, and that this effect was partially blocked by a 5-HT1A antagonist. Clark, Ashby, Dewey, Ramachandran, and Strecker (1996) found an increased DA response to cocaine in the NAC after an acute dose of FLX, however this did not reach significance, whereas an AMPH-induced accumbal dopaminergic increase was significantly enhanced by acute, but not chronic FLX pre-treatment (Ichikawa, Kuroki, & Meltzer, 1998; Sills, Greenshaw, Baker, & Fletcher, 1999). Concerning behaviour, Fletcher, Sinyard, Salsali, and Baker (2004) found that acute pre-treatment with FLX potentiated cocaine-induced locomotion.

Neurobiological studies support the neuropharmacological literature, showing that MPH-induced gene regulation, or "gene blunting" is potentiated in the striatum and NAC of rats who have been acutely co-administered MPH and FLX, compared to administration of MPH alone. Importantly, FLX had no effect by itself on the transcription factors (zif 268 and c-fos) that were measured in those areas (Steiner, Van Waes, & Marinelli, 2010; Van Waes, Beverly, Marinelli, & Steiner, 2010; Van Waes, Vandrevala, Beverly, & Steiner, 2014). More

recently it was found that not only did FLX potentiate the MPH-induced increase of 5-HT1B receptors in the striatum, but also that the 5-HT1B agonist CP94253 co-administered with MPH mimicked the effect of FLX, enhancing zif 268 induction in the striatum (Van Waes, Ehrlich, Beverly, & Steiner, 2015). This supports the findings of Borycz et al. (2008) described above.

This brings us to the human literature. A number of studies have looked at the effects of co-administering SSRI's and MPH in humans, with the majority of them examining the ability of MPH to accelerate and enhance the alleviation of depressive symptoms by SSRI's (Lavretsky, Park, Siddarth, Kumar, & Reynolds, 2006; Stoll, Pillay, Diamond, Workum, & Cole, 1996). The few that have investigated this topic in the context of ADHD have found no alteration of MPH-induced improvement in ADHD symptoms when adults (Biederman, Mick, Spencer, Surman, & Faraone, 2012) and children (Gammon & Brown, 1993) have been co-administered SSRI's with MPH, and have deemed the combination to be safe and lacking in adverse side effects (Findling, 1996). However these studies have taken the global clinical presentation of ADHD as their measure of improvement or severity of ADHD, utilising self-reported and/or observational general symptom scales that do not reveal the specific cognitive-behavioural deficits underlying the disorder. No study, to my knowledge, has looked specifically at objective cognitive measures of impulsivity, a process that is neurochemically distinct and dissociable from other processes affected by ADHD such as attention.

Given the extensive evidence that increased DA activity in the NAC is linked with increases in action restraint-type impulsivity, it is highly possible that this behavioural trait may be worsened by co-administration of SSRI's and MPH whilst the overall clinical presentation may not appear to be. The current study, among other goals, aims to show preclinical support for this hypothesis.

1.10. Aims and Hypotheses of the current study

1.10.1. Go/No-Go experiment

The aims of this study are nested in several layers. The first is to test a range of MPH doses on rats trained to perform a Go/No-Go task, measuring the 'action-restraint' subtype of impulsivity, as the vast majority of previous studies on rats have looked at the effects of this

drug on impulsivity as measured by the 5-CSRTT. To my knowledge, there has not been a MPH dose-effect study conducted on rats using the Go/No-Go task to measure action restraint, which is the paradigm (often couched within the more attention-demanding CPT) used to measure action restraint in humans. Participants are required to quickly select between either making a 'go' response or withholding that response based on the stimuli that is presented to them. The 5-CSRTT differs in that animal must always make a 'go' response in every trial, it is required to withhold it only until one of several 'go' signals is presented. It measures the animal's ability to wait to make the response, whereas the Go/No-Go, and most versions of the CPT, measure the subject's tendency to make an 'automatic' pre-potent response in a context in which it is inappropriate.

This could explain the discrepancy between the human and animal literature- that low doses of MPH improve action-restraint type impulsivity in humans, but studies on rodents have mostly failed to show this effect. It is predicted that using the Go/No-Go paradigm it will be found that, similar to humans, a low, clinically relevant dose of MPH (0.5 mg/kg) will increase rats''no-go' accuracy which is interpreted as a lowering of impulsivity, whereas higher doses will decrease this score, implying increased impulsivity.

Additionally this study will be looking at the effects of FLX on rats' performance in the go/no-go paradigm. Consistent with the finding that globally decreasing 5-HT levels in rats and humans increases impulsivity in action restraint paradigms, several studies have found that increasing 5-HT levels in rats via SSRI administration actually decreases impulsivity in the 5-CSRTT. It would be worthwhile replicating these results using a different paradigm. It is hypothesised that the dose found to be effective at reducing PR in previous studies (1 mg/kg) will decrease commission errors in the task, supporting the previous findings in the 5-CSRTT.

This brings us to the major goal of this study; to investigate the hypothesis that co-administering the SSRI FLX with a low dose of the psychostimulant MPH will decrease rats' 'no-go' accuracy in the Go/No-Go task, this effect being the opposite of the predicted effect for low-dose MPH, or FLX administered alone. This finding would have important clinical implications concerning the prescribing of MPH and FLX in combination for comorbid disorders such as ADHD and anxiety or depression.

1.10.2. Stop-signal reaction time (SSRT) experiment

A further aim of this study is to trial a new, simpler variant of the SSRT suitable for rodents. The SSRT is designed to measure the action-cancellation subtype of impulsivity, and was first developed to assess this cognitive-behavioural construct in humans by Logan, Cowan and Davis (1984). Their method was based on their 'racehorse' model of response inhibition, which proposes that the 'go' and 'stop' processes are separately controlled and in competition with one another. The closer the temporal proximity of the 'stop' signal is to the 'go' signal, the more likely the 'stop' process will 'win' the race, in which case the response is successfully inhibited. If the 'stop' signal occurs closer to the end of the 'go' process, there will not be enough time for the 'stop' process to be carried out before the 'go' process wins the race. By varying the time between the presentation of the 'go' and 'stop' signals, and recording the distribution of 'go response' reaction times, the individual's mean stopping latency can be estimated using a mathematical formula. A 'stopping function' can then be produced which plots the probability of the individual successfully inhibiting the go response, as a function of the time between the presentation of the 'stop' and 'go' signals. This function can then be compared between experimental conditions.

Eagle & Robbins (2003a) developed the first rodent version of the SSRT, which adheres closely to the original. Rats learn to perform a two part action, pressing a lever on presentation of a 'go' signal, which then causes a second lever to extend, which the rat presses to receive reinforcement. Rats then learn that if a 'stop' signal appears after the pressing of the first lever, they must not press the second lever. Successful inhibition of the second lever-press results in reinforcer delivery. Then, as described above, the time between the pressing of the first lever and the presentation of the 'stop' signal is varied and a stopping function is mapped. There are several things we wanted to simplify in our version of the paradigm. Aside from the complicated estimation and analysis of stopping latencies in this paradigm, the estimation of the stopping latency is completely reliant on the 'go' reaction time, meaning that general speeding effects are not dissociated from specific effects on action cancellation. If a particular treatment induced a general speeding or slowing effect then it would induce a corresponding change in stopping latency, which would not necessarily be indicative of a true change in impulsivity. Also, an individualised limited hold must be calculated for each rat, that is, the time that the second lever is extended. Due to individual variation in abilities, having the same LH for all rats would make the task too easy for some and too difficult for others. This measure had to be taken to achieve an optimum level of

accuracy for each rat. This could prove problematic if some rats improved at the task faster than others, due to a practice effect for example.

The SSRT task that was designed for this experiment requires no estimation of stopping latency or complicated formulas as stopping times are measured directly, all rats can perform with same task parameters, and the 'go' RT is independent of the stopping latency. However a new variable, response rate, can influence stopping latency as a result of general speeding or slowing, necessitating it's analysis alongside stop latency.

A selection of MPH doses will be administered to rats performing this task in order to validate the unique variant of the SSRT developed for this investigation. Replication of past results that have found an impulsivity-reducing effect of MPH in this type of paradigm would indicate that it is likely to be measuring the same construct.

An additional goal is to add further support for the previously observed dissociation between action restraint and action cancellation in terms of 5-HT involvement. In this regard, FLX will be administered to rats performing the SSRT task. It is expected that unlike the hypothesis for the Go/No-Go task, FLX will not have no significant effect on rats' latency to stop responding, which translates as the ability to cancel an already initiated sequence of actions.

Unlike the prediction for the Go/No-go task, It is predicted that co-administration with FLX will not attenuate the impulsivity-reducing effect of MPH for rats performing the SSRT variant, as manipulations of DA activity have failed to have an effect on action cancellation in past studies. An exception must be noted though- Eagle et al. (2011) found that blocking D2 receptors in the striatum increased stopping latency, while striatal D1 receptor antagonism speeded it. However as DA increase in the striatum was not potentiated by co-administration with FLX (Weikop et al., 2007b), this observation does not bear on our hypothesis.

There is a possibility that the potentiated increase in accumbal NE seen with the combination of citalopram and MPH (Weikop et al., 2007b) could in fact result in an improved performance in the SSRT, based on the stop-latency decreasing effects of NET inhibitors (see section 1.5). Although the effects of ATX on NE levels in the NAC have not been investigated, it is possible that levels are raised here much like they are in the PFC, potentially contributing to its ameliorative effects on impulsivity.

1.10.3. Locomotor activity experiment

The association between DA levels in the NAC and motor activation has been thoroughly researched, with various types of studies demonstrating that manipulations resulting in increased accumbal DA outflow also induce an increase in locomotor activity (e.g. Boye, Grant, & Clarke, 2001; Hedou, Feldon, & Heidbreder, 1999; Oades, Taghzouti, Rivet, Simon, & Le Moal, 1986). Without measuring DA levels directly, this is the best behavioural paradigm to assess whether the combination of MPH and FLX, like in the studies of Weikop et al. (2007a, 2007b) is increasing accumbal DA levels beyond the increase induced by MPH alone.

To investigate this, open-field activity will be assessed after MPH and FLX alone, and in combination, with the prediction that activity will be significantly higher after the combination relative to the two drugs alone, in line with the data from Borycz et al. (2008). If the predicted effects on action restraint do occur, this finding would provide support that they are likely due to the potentiation of DA release as previously observed.

2. Method

2.1. Animals

Thirteen male Sprague-Dawley rats, bred in the VUW School of Psychology Animal Facility, were used for the Go/No-Go task, and thirteen male Wistar rats, also bred in the Animal Facility, were used for the Stop Signal Reaction Time Task (SSRT). Twenty-five of these rats were used for the locomotor experiment, (12 of the Sprague-Dawleys and all 13 of the Wistars). At the commencement of training for their respective tasks the rats were four months old. Throughout the training and experimental period rats were food restricted to maintain a target weight of around 85% of their free-feeding bodyweight, however one week prior to the locomotor experiment they were put on free-feeding, and had access to unrestricted food throughout that experiment. Their diet was comprised of Purina standard laboratory chow, and they had continuous access to water in their home cages. Rats were pair-housed, unless one of the pair became too dominant and put on weight at the expense of the other rat- in this case, the 2 paired rats would be housed singly in order to bring them back to their target weights, after which they would be returned to their original cage. Rats

were kept on a reverse light-dark cycle, with lights controlled by an automatic timer coming on at 7 am and turning off at 7 pm.

2.2. Equipment

2.2.1. Go/No-Go and SSRT

Thirteen operant chambers obtained from Med Associates Inc. were used, one per rat for each task. Each chamber was made of clear plastic, with a floor made of metal grating. The chambers measured 31.8 cm x 25.4 cm x 26.7 cm. On the right-hand inside wall were three retractable levers with lights above each. A magazine situated in the bottom middle of this wall received sugar pellets from a pellet dispenser on the outside of the chamber. The operant boxes were controlled by two computers running Med Associates software, on which the Go/No-Go and SSRT programmes were written.

2.2.2. Locomotor activity

Experimental sessions were carried out using 13 activity-monitoring open-field chambers (Med Associates Inc., USA; model ENV-515) in an air-conditioned room. Chambers were set inside sound attenuating cupboards, the doors of which were closed throughout the experiment. The floor space of the chambers measured 42 x 42 cm, with clear plastic walls 30 cm high. The floor was light-coloured smooth plastic. The area of the chamber was divided by 16 vertical and horizontal infa-red beams forming a grid, with squares measuring 25 x 25 mm. Animals' movement was recorded as they crossed the beams, with this information being fed to computers which recorded the information using Med Associates software.

2.3. Drugs

0.9% saline was injected intraperitoneally (i.p.) at a volume of 1 ml/kg, this dose served as the control baseline condition. Methylphenidate was obtained in powder form from Barry Dent Industries, Porirua, Wellington. It was dissolved in 0.9% saline to whichever mg/ml concentration was required, and administered to the rats via i.p. injection at a volume of 1 ml/kg. Fluoxetine was obtained in tablet form, as the commercially available antidepressant (Pharmachemie, Haarlem, the Netherlands). A solution was made by crushing

the tablets finely and mixing them with 0.9 % saline (with 2% tween) using a vortex. This solution was then left on a stir plate overnight to aid with the dissolving of the powder. Fluoxetine was injected at a volume of 1 ml/kg, i.p. Drug solutions were refrigerated for the duration of their use in the experiment, and discarded after 1 month.

2.4. Go/No-Go Task

2.4.1. Training

2.4.1.1. Autoshaping

Each rat was placed in its own operant chamber. During these sessions the left lever was extended, with the light above it turned on. If no response was made on the lever after its 'limited hold' (LH) of 15 s, it was retracted, the light went off, and a 'free' sugar pellet was delivered. After 30 seconds, the lever would again be extended and the light above it illuminated. This sequence of events repeated continuously for 30 minutes. If the lever was pressed during the 15 s that it was extended, it was withdrawn and a sugar pellet was delivered. If rats made 10 responses in a session, no more 'free' sugar pellets were delivered. If rats were not making responses after three sessions of autoshaping, the time between 'free' pellets was extended. When all rats were making >15 responses they were put onto '10% no-go' training (one did not reach this stage and so was excluded from further training).

2.4.1.2. 10% No-go training

These sessions followed the same format as the eventual Go/No-Go experimental sessions, however they were comprised mostly of 'go' trials, in which the left lever was extended for a limited hold of 6 s and the light above it illuminated. A press on the lever resulted in the lever retracting, light turning off, and pellet delivery, with the next trial beginning after 5 s (giving time for the rat to consume the pellet) plus an ITI that was calculated based on latency, using the formula $ITI = 15 - latency / 100$. If the lever was not pressed in the 'go' trial, it was retracted and light was extinguished for the length of the ITI only. During 'no-go' trials, which made up 10% of total trials in the session, the left lever was extended for 6 s however the light above it was not illuminated. Instead, the light above the right lever, which itself remained retracted, was illuminated. In these trials, the rewarded behaviour was reversed- a press on the lever resulted in the lever retracting and no pellet delivery, whereas no response on the lever during its LH resulted in the lever retracting and pellet delivery. The 'go' and 'no-go' trials were interspersed in a random order, with the

session ending once rats had completed 120 trials, or after 50 minutes. Once rats were successfully responding in >50% of the go trials, they were moved on to the experimental task in its final form. Four of the original cohort were replaced by new rats after not meeting this criteria around three weeks after all the other rats had. Due to the new rats' late arrival and need to be trained, they started drug sessions later than the original nine.

2.4.1.3. Go/No-Go training

These sessions were the final version of the task, to be used during the experimental drug sessions. The number of 'no-go' trials was increased to 60, to make up 50% of all trials. 40% of trials retained the original 6 s LH, while 40% had a longer LH of 9 s, and 20% had a shorter LH of 3 s. (fig.1, p. 44) Any given trial combined one of the trial type options with one of the limited hold options, with the order of trials of different types being randomly generated, but the overall proportion of each category being held constant. When all the rats had reached a relatively stable level of performance, where no identifiable trend in scores could be observed and scores did not vary by 10% for three consecutive days, as well as having >70% success on the go trials, they began on the experimental phase.

2.4.2. Experimental phase

2.4.2.1. Design

Drug sessions were conducted twice a week, on either a Tuesday and a Friday or a Wednesday and a Saturday, with training sessions on the other weekdays. The sessions occurred between 9 am and 1 pm. Refer to Table 1(p. 50-51) for the order of drugs/doses and number of sessions conducted.

2.4.2.2. Drug sessions

Animals were weighed before being placed in their respective chambers and then injected. In the conditions requiring two drugs to be administered, the rats would receive injections to opposite sides. The task would begin for each rat 15 minutes after they were injected, with the programme being loaded individually for each rat in a sequential fashion, timed by the experimenter. When the last rat had finished the task by completing 120 trials, all rats were returned to their home cages and fed.

2.4.3. Dependent variables

2.4.3.1. 'Go' accuracy

The 'go' score was calculated by dividing the number of successful go trials ('go' trials in which the lever was pressed) by 60 (the total number of go trials). This was calculated overall, and separately for each of the three LH's.

2.4.3.2. 'No-go' accuracy

The 'no-go' score was calculated by dividing the number of successful no-go trials ('no-go' trials in which the lever was not pressed) by 60 (the total number of no-go trials). This was calculated overall, and separately for each of the three LH's.

2.4.4. Independent variables

2.4.4.1. Drug treatment/dose

MPH was administered initially in a subgroup of the rats in doses of 0.5, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/kg, but in further rats only the 0.5, 2.5, and 7.5 doses were administered. FLX was administered in doses of 1.0 and 5.0 mg/kg, and two of the conditions combined the 0.5 mg/kg MPH dose with the 5.0 mg/kg FLX dose, and the 2.5 mg/kg MPH with the 5.0 mg/kg FLX dose. Saline sessions were used as baselines for behaviour.

2.4.4.2. Lever latency

This variable refers to the length of the limited hold of the lever in the trial, either 3 s, 6 s or 9 s. The 3 s latency was intended to be the 'easiest' for the 'no-go' trials, as it allowed the least time in which the rat could make a response (error). Whereas, for the 'go' trials, the 3 s latency was intended to be the most 'difficult', as responding in these trials was correct, and little time was given to make a response. The opposite was true for the 9 s latency trials.

2.4.4.3. Baseline impulsivity

This between-subjects variable divided rats into two groups- 'high impulsivity' (HI) and 'low impulsivity' (LI). Assignment to either group was determined with a median-split analysis using the average 'no-go' accuracy scores from the first set of saline baseline sessions. The scores were ranked from lowest to highest (low 'no-go' accuracy reflecting high impulsivity in this paradigm), and the half with lower scores were labelled LI, while the half with higher scores were assigned to the HI group. In the case of an odd number of rats in

the group, as was the case in most of the analyses (n = 13), the rat with the median score was assigned to the group with the mean score that was closest to its own score. This happened to be the HI group, making the HI group n = 7 and the LI group n = 6, apart from the analysis on the FLX doses administered alone, where both groups numbered 6.

2.5. SSRT task

2.5.1. Training

2.5.1.1. Autoshaping

Autoshaping followed the same procedure as outlined in Go/No-Go section.

2.5.1.2. 'Go' signal training

After autoshaping was completed, rats were placed into phase 1 of 'go' training, where they learned an FR3 response. At the beginning of each trial, the left lever would extend and the light above it would be illuminated. In order to receive a sugar pellet, the rat needed to press the lever three times within the lever's LH of 60 seconds. If it did so, the lever would retract, the light above it would turn off, a pellet would be delivered with 5s allowed to for the rat to consume it, and 10 seconds ITI would elapse before the next trial began (fig. 2a, p. 45). If the rat did not press the lever, or pressed it less than three times before the LH was over, the lever retracted, the light above it would turn off, and no reinforcer was delivered before the 10 s ITI (fig 2b, p. 46). When rats had passed criteria (>20 successful trials in a session) they were introduced to phase 2. These sessions were the same as the first, however a second trial type was introduced, in which rats had to press the left lever six times in order to earn a pellet (FR6). These trials made up 80% of the session, and were randomly interspersed with the FR3 trials which made up the remaining 20%. When rats were completing >20 of the FR6 trials successfully, they were moved on to phase three, in which a third, 9-press trial type was introduced. The FR9 trial made up 80% of the total trials, with 10% FR3 trials and 10% FR6 trials (all interspersed randomly). When rats had reached criteria on the FR9 trials, they were deemed to be fully 'go' trained, and were moved onto training in the final version of the task.

2.5.1.3. 'Stop' signal training

These sessions were the final version of the task, and included all three types of go trial (FR3, FR6, FR9) whilst introducing a new set of 3 different 'stop' trials, distributed equally to make up 50% of the total number of trials. These trials mirrored the 'go' trials, in that they began the same way, eliciting the same pressing response on the right lever. However in the 'stop' trials a new stimulus was introduced (the 'stop signal'), which consisted of the light above the left lever becoming illuminated, and the light above the right lever turning off. This would occur immediately following what would be the penultimate press had it been a 'go' trial. Thus, the stop signal could appear after either 2, 5 or 8 presses. The six types of trial occurred in random order throughout the session.

There were several possible outcomes of the 'stop' trials, depending on the rat's actions. If a rat kept pressing on the lever after the stop signal was displayed, the right lever would eventually retract after the LH of 60 seconds, the left 'stop-signal' light would turn off, and the next trial would begin after an ITI of 10 seconds (fig. 2c, p. 47). If the rat withheld from pressing for a full 3 s after the presentation of the stop signal, any time within that 60s period (even if it had made lever presses after the stop signal appeared) the lever would retract, light turn off, and a pellet would be delivered (fig. 2d, 2e, p. 48-49). Trials of all 6 types had equal chance of occurring, and were interspersed randomly throughout the session.

Rats were trained on this task until they showed relatively stable performance, with no obvious trending of scores upwards or downwards, and with success in >70% of the go trials.

2.5.2. Experimental phase

This followed the same format as the Go/No-Go group's experimental phase, using the same version of the SSRT task that was used during stop-signal training. Refer to Table 2 (p. 51) for the order of drugs/doses and number of sessions conducted.

2.5.3. Dependent variables

2.5.3.1. Stopping latency

The length of time it took the rat to stop responding after presentation of the stop signal. It was represented by the length of time (measured in hundredths of a second, and represented by the unit '$s/100$') between the stop signal appearing and pellet delivery after rats withheld responding for 3 seconds, averaged over all stop trials in a session, and then over the

2-3 sessions conducted for each particular treatment. For trials in which the animal successfully made no responses after presentation of the stop signal, their latency would measure $300_{s/100}$. Animals were given an average score, (mean for all stop trials) and also separate scores for each of the stop trial types (FR2, FR5 and FR8).

2.5.3.2. Response rate

To give a more detailed picture of how the rats were responding after the stop signal appeared, their response rate was also calculated. This was simply their average latency for the session divided by the average number of extra responses that were made after the stop signal appeared. This value was then averaged over all sessions conducted under the same treatment, giving an average response rate for that treatment. This measure allowed a dissociation between changes in latency that were due to a general speeding or slowing of response rate, and changes that were possibly due to the variable in question- the ability to cancel an action. For trials in which the animal successfully stopped, i.e. made no extra responses after presentation of the stop signal, the rate could not be calculated. In these cases, the data from that particular session was omitted from the calculation of the average response rate for that drug/dose (as each treatment was administered in 2-3 sessions). Again, this was calculated for each animal as an average over all trials as well as separately for each stop trial type.

2.5.4. Independent variables

2.5.4.1. Drug treatment/dose

MPH was administered in doses of 0.5, 1.0, 2.5, 5.0, 7.5 mg/kg. FLX was administered in doses of 1.0 and 5.0 mg/kg, and three of the conditions combined either the 0.5, 1.0 or 2.5 mg/kg MPH dose with the 5.0 mg/kg FLX dose. Saline sessions were used as baselines for behaviour.

2.5.4.2. FR (fixed response)

The number of lever presses required to receive reward (in go trials) or for the stop signal to appear (in 'stop' trials). The FR types for go trials were FR3, FR6 and FR9. The types for 'stop' trials were FR2, FR5 and FR8. Each of the trial types had an equal chance of occurring, and it was hypothesised that the more presses the rat had to make before the stop signal occurred, the more difficult it would be for it to stop due to the response rate having

increased over the course of making a long sequence of responses in order to obtain a reward (as 'go' and 'stop' trials cannot be differentiated until either the reward or the stop signal appear).

2.5.4.3. Baseline impulsivity

A median split (see section 2.4.4.3) created two groups of rats based on average stopping latency for the saline sessions. The high impulsivity group (HI) consisted of the rats with the slower stopping latencies (n = 7) and the low impulsivity group (LI) consisted of the rats with the faster stopping latencies (n = 6).

2.6. Statistical analyses for the Go/No-Go and SSRT

The Statistical Package for the Social Sciences (SPSS, v. 20) was used for all analyses. Alpha level was set at $p \leq .05$. Separate mixed ANOVAs were run on 'no-go' accuracy and 'go' accuracy (or stopping latencies) for the MPH doses and the FLX doses, including the additional factors baseline impulsivity (between-subjects) and lever latency/trial FR (within-subjects). These analyses were performed in order to determine if there were any main effects of these factors, as well as to determine whether these factors interacted with any treatment effects induced by the drug. It was possible that ceiling effects in the group of animals with high baseline 'no-go' accuracy or a fast stopping speed (LI) may have masked MPH-induced improvement in a whole-group analysis, or counter to that, a floor effect may have been seen with the HI group, masking doses of MPH that may have been decreasing 'no-go' accuracy or increasing stopping latency for the HI rats. A similar effect may be seen in lever latency/trial FR, whereby the easier trials may mask ameliorative drug effects, whereas the difficult trials may uncover these effects. The opposite would be true of detrimental drug effects. Any interactions would be analysed using separate one way repeated measures ANOVA to determine how treatment effects varied between groups or trial type/s, and any main effects would be analysed using post-hoc t-tests with a Bonferroni corrected alpha level for multiple comparisons.

For the MPH treatments, which had two saline baselines, the second saline session was not included. Having the baseline measure at the second time-point may have artificially obscured or exaggerated interactions between variables. Thus, once any interactions had been analysed, a final one-way ANOVA was carried out on the group or trial type/s that showed treatment effects.

With regards to 'no-go' accuracy, when comparing the effects of MPH and FLX administered alone to their effects when administered together as one treatment, t-tests would be used without a Bonferroni correction, as this is acceptable when analysing results pertaining to a priori hypotheses. However when comparing the effects combined and single treatments on 'go' accuracy scores, for which no prior hypotheses were made, repeated measures ANOVA were used, followed up by post-hoc Bonferroni-corrected t-tests if a main effect was found.

For the SSRT, as the baselines differed for the different treatments, to compare the combined treatments directly with either of the single treatments required a comparison of the treatments' percentage change from baseline. This was performed if effects of the combined treatment on stopping latency differed from those of the drugs administered separately.

All ANOVA statistics were reported using the Greenhouse-Geisser correction if the particular data set violated the assumption of sphericity (Mauchley's test).

2.7. Locomotor activity
2.7.1. Procedure

Rats were brought into the locomotor room from their housing room, weighed, and placed in their chamber (rat-chamber pairings did not vary over experimental sessions). Lights were turned off apart from a dim red bulb, and a white noise generator was turned on. Animals were left to habituate for 30 minutes. Each animal was then taken out of its chamber and injected intraperitoneally with its assigned drug treatment then placed back into the chamber, with recording of movement beginning 5 minutes after the injection(s) and continuing for 70 minutes. It took around 20 minutes to inject the whole group, but as recording was manually started for each animal separately, the recorded window was consistent across all animals. When the 70 minutes was over for the last animal to be injected, all animals were removed from the chambers and taken back to their home cages. Chambers were cleaned thoroughly with Virkon 'S' disinfectant (Southern Veterinary Supplies, NZ) after each locomotor session, to remove any odours.

2.7.2. Design

Seven sessions were run, each two days apart, with the first being a 75 minutehabituation session for which activity was not recorded and no injections were given. The following six sessions were all drug sessions during which all animals were injected with one of five drug treatments, or saline, according to a counterbalanced schedule (table 3, p. 52-54).

2.7.3. Dependent variables

2.7.3.1. Ambulatory counts

The sequential breaking of four beams (in any part of the chamber) was recorded as one ambulatory count. All measures of activity began exactly five minutes after the animal was injected. This was to eliminate any activity-related after-effects of injection stress that may have varied between rats and treatments.

2.7.4 Independent variables

2.7.4.1. Drug treatment/dose

The six treatments given were saline, 0.5 mg/kg MPH, 2.5 mg/kg MPH, 5.0 mg/kg FLX, 0.5 mg/kg MPH + 5.0 mg/kg FLX, and 2.5 mg/kg MPH + 5.0 mg/kg FLX.

2.7.4.2. Time

Activity was recorded for a total of 70 minutes, with the measures being divided into time bins of 5 minutes, giving a total of 14 time-points over the course of the session. Each time-point reflected the sum of ambulatory counts made over the previous five minutes, for example, the number of ambulatory counts at the 20 minute time point was the number made during minutes 15 – 20.

2.7.5. Statistical analysis

The Statistical Package for the Social Sciences (SPSS, v. 20) was used for all analyses. A 6 x 14 repeated-measures ANOVA was run on the data to determine the effects of drug treatment (6) and time bin (14) on number of ambulatory counts. In the case of an interaction between the two factors, separate one-way ANOVAS were conducted, comparing the effects of the six drug treatments for different time bins or combinations of time bins.

These analyses would determine if there was an effect of any of the treatments on activity at any particular time point. Any ANOVAs showing a significant treatment effect would be re-run to include the between-subjects factor 'breed of rat' to examine whether there was an interaction with treatment effects.

Bonferroni- corrected post-hoc t-tests would be conducted on any ANOVAs showing a significant effect, in order to determine whether administering a combination of FLX and MPH resulted in higher activity than either of the drugs administered alone, for either of the two MPH doses.

Finally, each rat's difference to baseline for total ambulatory counts during minutes 5-35 in several of the drug conditions was calculated, then correlated (using a Pearson's correlation) with change from baseline in no-go accuracy to investigate whether there was any relationship between locomotor elevating effects of drug treatment and effects on action restraint.
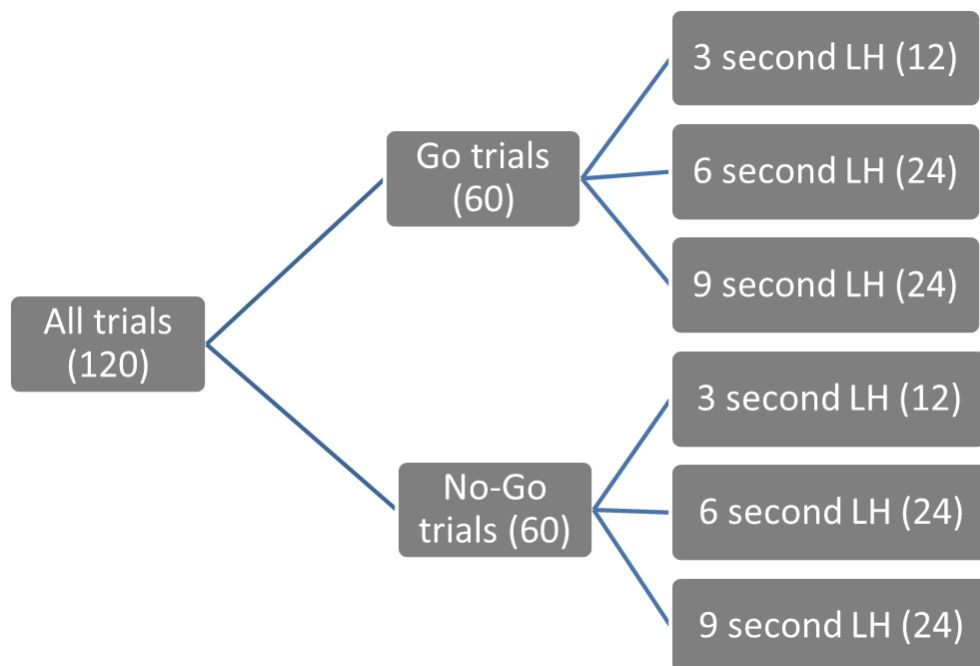
Figure 1. Composition of trial types in final version of Go/No-Go task.
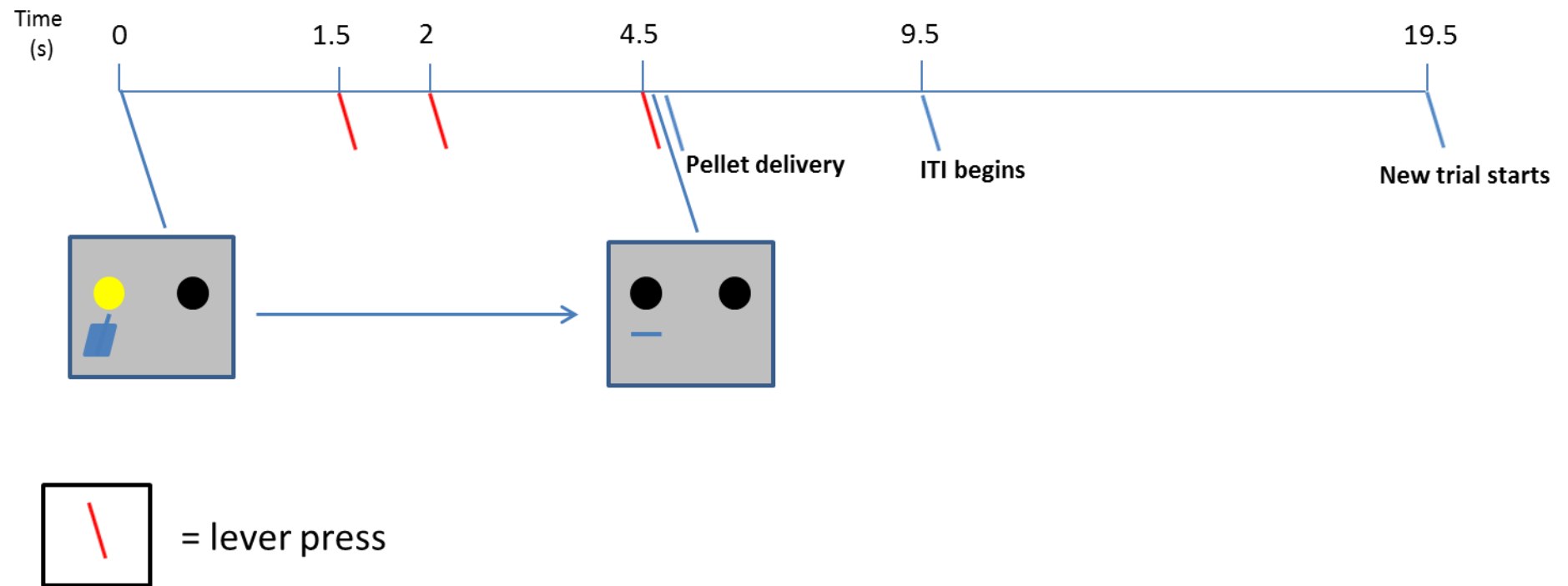
Figure 2a. An example of a successful FR3 'go' trial. Reinforcer delivered for pressing lever 3 times within 60s LH.
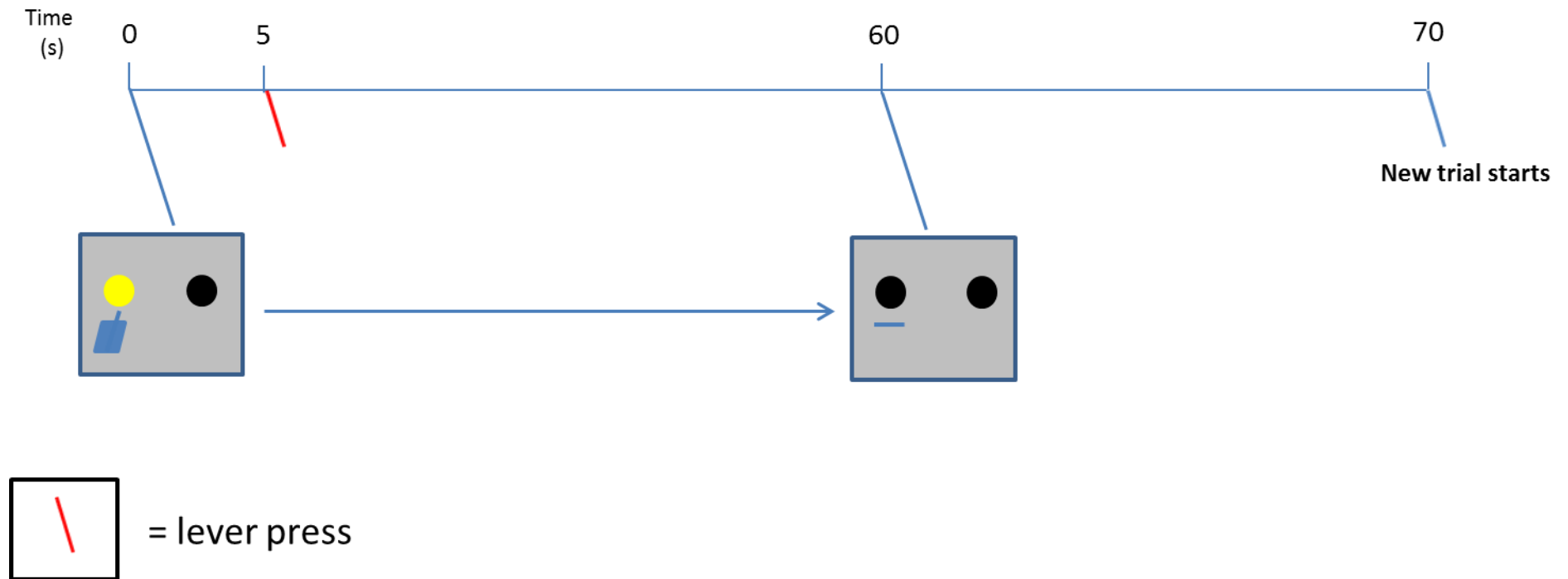
Figure 2b. Example of an unsuccessful FR3 'go' trial. The lever has not been pressed the required 3 times within the 60s LH, no reinforcer is delivered. Alternatively this could be an FR2 'stop' trial in which the trial is cancelled before the stop signal is presented as the lever has not been pressed the required 2 times within the 60s LH in order for the stop signal to appear.
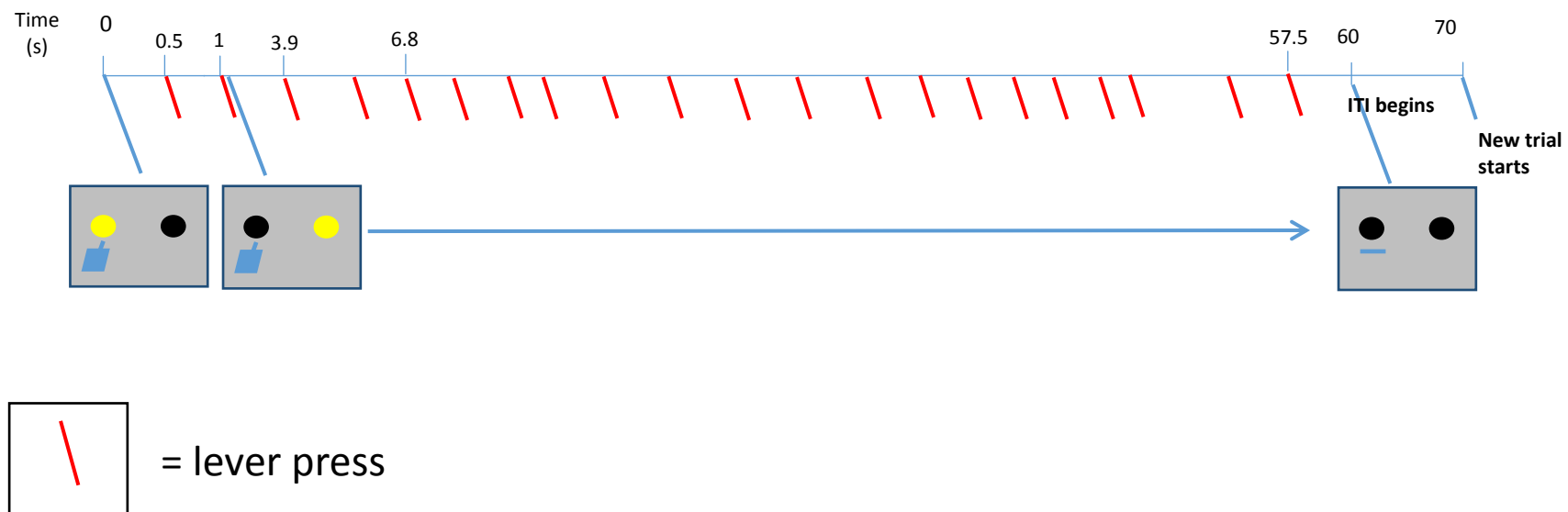
Figure 2c. Example of an unsuccessful FR2 'stop' trial. Lever is pressed the required 2 times in order for the stop signal to be presented. In this example however the rat continues to press the lever at intervals < 3 s for the duration of the 60s LH. No reinforcer delivered. Animal's stopping latency is recorded as 6000 *s/100*.
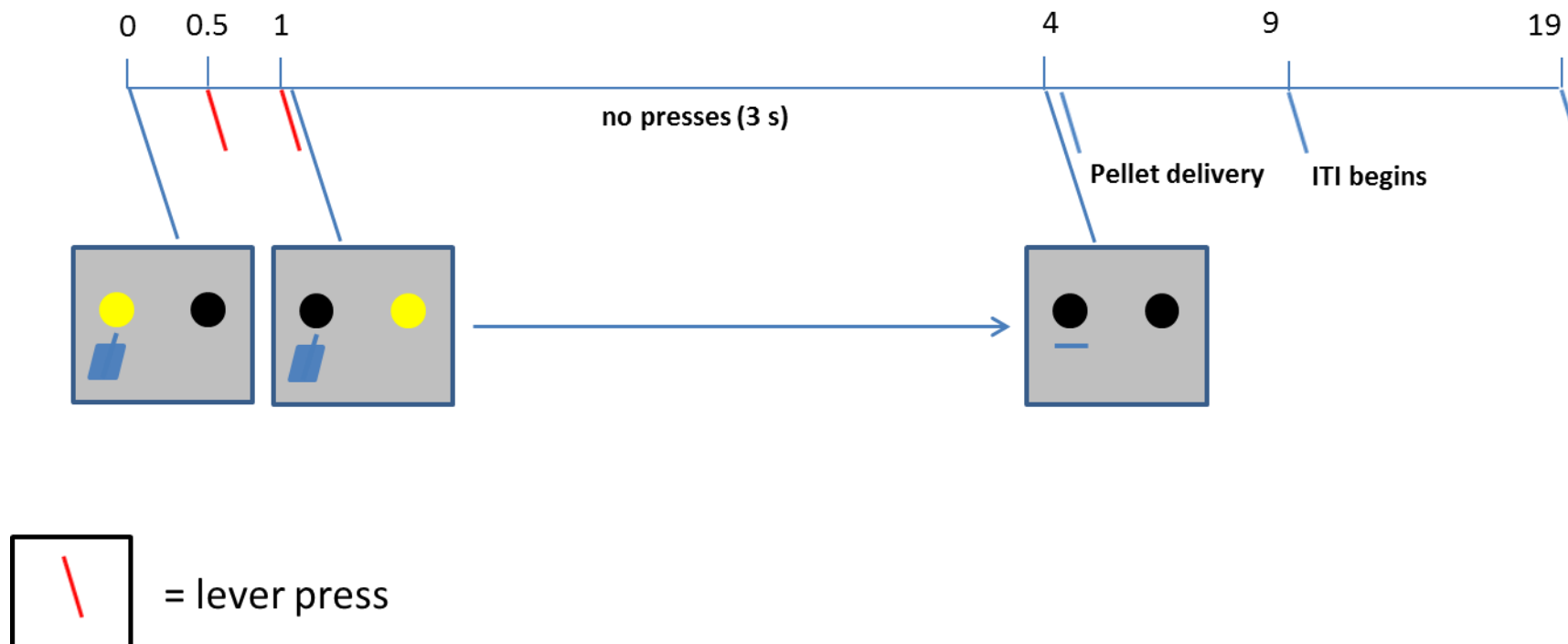
Figure 2d. Example of a successful FR2 'stop' trial in which no lever presses are made after the presentation of the stop signal. Reinforcer is delivered, animal's latency is recorded as 300 *s/100*.
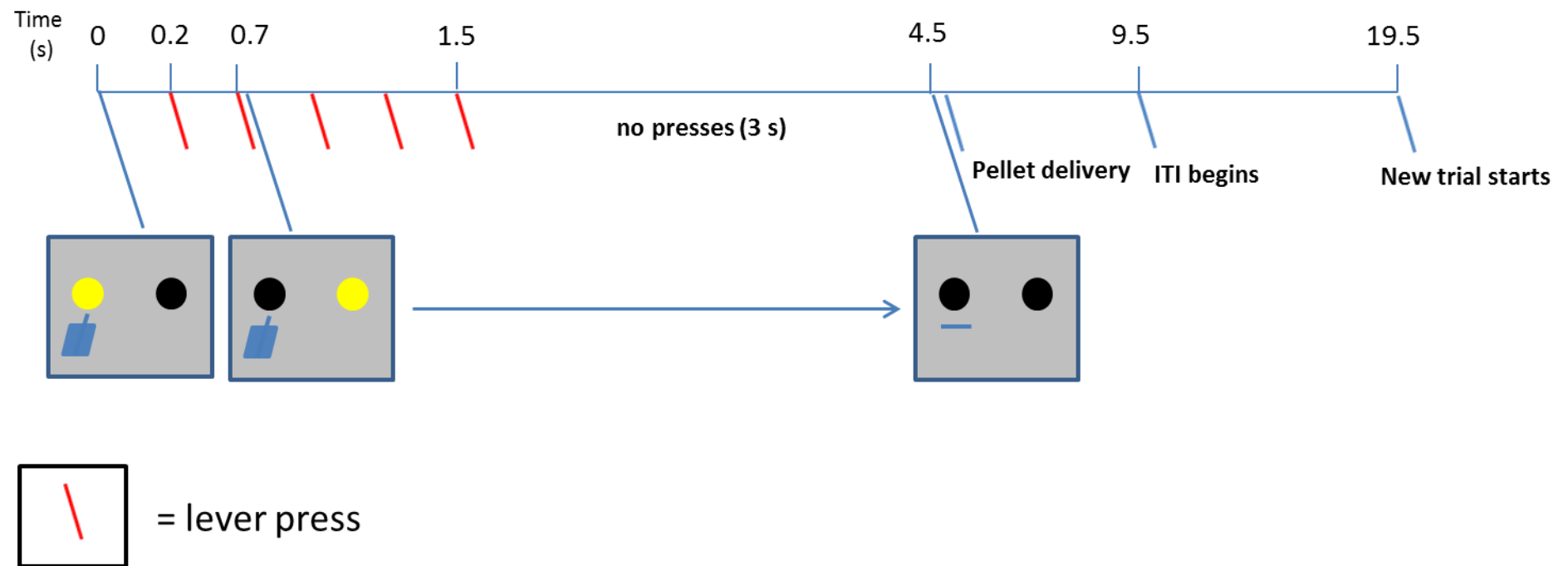
Figure 2e. Example of a successful FR2 'stop' trial. Lever presses are made after presentation of the stop signal, but animal manages to refrain from pressing the lever for 3 s within the 60 second LH. Reinforcer is delivered. Animal's latency is recorded as 380 *s/100*.

	1	2	3	4	5	6	8	9	10	11	12	13	14	15
11 14 22 26	Saline #1	0.5 mg/kg MPH	5.0 mg/kg FLX	0.5 mg/kg MPH + 5.0 mg/kg FLX	2.5 mg/kg MPH	7.5 mg/kg MPH	2.5 mg/kg MPH + 5.0 mg/kg FLX	1.0 mg/kg FLX						
	3	4	2	3	2	2	2	2						
13 15 16 17 18 21	Saline #1	0.5 mg/kg MPH	1.0 mg/kg MPH	2.5 mg/kg MPH	5.0 mg/kg MPH	10.0 mg/kg MPH	7.5 mg/kg MPH	Saline #2	2.5 mg/kg MPH + 5.0 mg/kg FLX	0.5 mg/kg MPH + 0.5 mg/kg FLX	Saline #3	0.5 mg/kg MPH #2	5.0 mg/kg FLX	1.0 mg/kg FLX
24 25	4	3	3	3	3	1	2	2	3	2	2	2	2	2
23	Saline #1	0.5 mg/kg MPH	1.0 mg/kg MPH	2.5 mg/kg MPH	5.0 mg/kg MPH	10.0 mg/kg MPH	7.5 mg/kg MPH	Saline #2	2.5 mg/kg MPH + 5.0 mg/kg FLX	0.5 mg/kg MPH + 0.5 mg/kg FLX	Saline #3	0.5 mg/kg MPH #2	5.0 mg/kg FLX	

	4	3	3	3	3	1	2	2	3	2	2	2	2	

Table 1: Treatment orders for go/no-go experiment. Individual rats numbered down left side, treatment order runs along the top. Number of sessions run below each treatment. This table gives no indication of timeline, treatments occurring in same column were not necessarily conducted at the same time point. Only meant to provide information on the order of conditions for each rat.

1	2	3	4	5	6	7	8	9	10	11	12	13
Saline #1	0.5 mg/kg MPH	2.5 mg/kg MPH	1.0 mg/kg MPH	7.5 mg/kg MPH	5.0 mg/kg MPH	5.0 mg/kg FLX	0.5 mg/kg MPH + 5.0 mg/kg FLX	Saline #2	2.5 mg/kg MPH + 2.5 mg/kg FLX	1.0 mg/kg FLX	2.5 mg/kg MPH #2	1.0 mg/kg MPH + 5.0 mg/kg FLX
3	3	2	3	2	2	2	2	2	2	2	1	2

Table 2: Treatment order for SSRT experiment. Treatment order runs across the top. Number of sessions run below each treatment. All animals experienced the same order of drug conditions.

rat	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6
11	Saline	MPH 0.5 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 2.5 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	FLX 5.0 mg/kg
13	Saline	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 2.5 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg	FLX 5.0 mg/kg
14	Saline	MPH 2.5 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg	FLX 5.0 mg/kg
15	FLX 5.0 mg/kg	MPH 2.5 mg/kg	MPH 0.5 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	Saline
16	FLX 5.0 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg	MPH 2.5 mg/kg	Saline
17	FLX 5.0 mg/kg	MPH 0.5 mg/kg + FLX	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 2.5 mg/kg	MPH 0.5 mg/kg	Saline
18	FLX 5.0 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg	MPH 2.5 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	Saline
21	FLX 5.0 mg/kg	MPH 0.5 mg/kg	MPH 2.5 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	Saline
22	FLX 5.0 mg/kg	MPH 2.5 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	Saline
24	FLX 5.0 mg/kg	MPH 0.5 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 2.5 mg/kg	Saline
25	Saline	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 2.5 mg/kg	FLX 5.0 mg/kg
26	Saline	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg	MPH 2.5 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	FLX 5.0 mg/kg
31	saline	MPH 0.5	MPH 2.5	MPH 0.5	MPH 2.5	FLX 5.0

		mg/kg	mg/kg + FLX 5.0 mg/kg	mg/kg + FLX 5.0 mg/kg	mg/kg	mg/kg
33	saline	MPH 2.5 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	FLX 5.0 mg/kg
34	saline	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 2.5 mg/kg	MPH 0.5 mg/kg	FLX 5.0 mg/kg
35	saline	MPH 0.5 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 2.5 mg/kg	FLX 5.0 mg/kg
36	saline	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 2.5 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg	FLX 5.0 mg/kg
37	FLX 5.0 mg/kg	MPH 2.5 mg/kg	MPH 0.5 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	Saline
38	saline	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 2.5 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg	FLX 5.0 mg/kg
41	FLX 5.0 mg/kg	MPH 2.5 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	Saline
42	FLX 5.0 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg	MPH 2.5 mg/kg	saline
43	saline	MPH 0.5 mg/kg	MPH 2.5 mg/kg	MPH 0.5 + FLX 5.0 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	FLX 5.0 mg/kg
44	FLX 5.0 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	MPH 0.5 mg/kg	MPH 2.5 mg/kg	MPH 0.5 mg/kg + FLX 5.0 mg/kg	Saline
45	FLX 5.0 mg/kg	MPH 2.5 mg/kg	MPH 0.5 + FLX 5.0 mg/kg	MPH 0.5 mg/kg	MPH 2.5 mg/kg + FLX 5.0 mg/kg	saline
46	FLX 5.0 mg/kg	MPH 0.5 mg/kg +	MPH 2.5 mg/kg	MPH 2.5 mg/kg +	MPH 0.5 mg/kg	Saline

		FLX 5.0 mg/kg		FLX 5.0 mg/kg		

Table 3. Treatment order for locomotor activity. Sessions were conducted two days apart.

3. Experiment 1: Go/No-Go results

The purpose of this experiment was to examine the effects of a range of MPH and FLX doses on 'no-go' accuracy (a measure of 'action restraint' type impulsivity) with the hypothesis that both the low 0.5 mg/kg dose of MPH and the 1.0 mg/kg FLX would improve this score, but that a combination of these two drugs would decrease no-go accuracy. Measures of 'go' accuracy were analysed alongside 'no-go' scores to give an indication whether changes in 'no-go' accuracy were truly reflective of a change in impulsivity, or more of a change in general arousal or attention.

3.1. Multiple baselines

Three separate phases of saline treatment were given at different time-points over the course of the experiment, to gauge whether baseline measures of 'no-go' accuracy were changing due to a practice effect. T-tests revealed that the differences in mean baseline 'no-go' scores across the three time-points did not reach significance, however as the saline #2 'no-go' accuracy baseline was much higher than saline 1 (fig. 3) it was decided that separate baseline measures would be used for different treatments based on proximity in the experimental timeline. Thus, saline #1 is used as the baseline comparison for MPH doses 0.5 mg/kg and 2.5 mg/kg, whereas saline #2 is used as the baseline comparison for the 7.5 mg/kg MPH dose. Saline #3 is used as the baseline comparison for the two FLX doses, and all of the combined drug treatments.
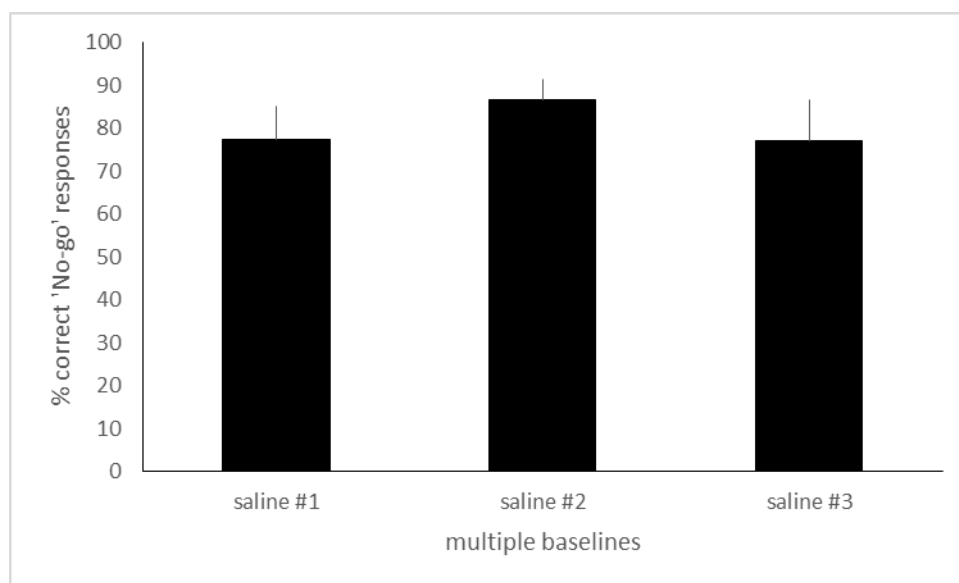
Figure 3. Mean percentage accuracy in the 'no-go' trials (± SEM) for the subgroup of rats (n=9) that began the experiment first and took much longer to complete all drug treatments, thus had baseline behaviour monitored at three time-points to gauge any practice effects. None of the saline baseline measurements of accuracy in no-go trials differed significantly from one another, however the second baseline accuracy measurement (86.75 ± 4.72)trended towards being significantly higher than the first (77.34 ± 7.76), t(8) = -1.947, p = .087, whilst the first and third (77.04 ± 9.58) baseline accuracy measures were very similar, t(8) = 0.042, p = 0.97. The difference between the second and third baseline accuracy measures was also not significant t(8) = 1.62, p = .13.

3.2. Effects of MPH alone

3.2.1. 'No-go' accuracy

3.2.1.1. Influence of lever latency and rats' individual baseline impulsivity on the effects of MPH on 'no-go' accuracy.

MPH doses chosen to be included in the analysis were:

1) 0.5 mg/kg, a low, clinically relevant dose, which is hypothesised to improve 'no-go' accuracy.

2) 2.5 mg/kg, as this is the dose that Weikop et al. (2007a, 2007b) demonstrated to have its effects on DA levels potentiated by FLX.

3) 7.5 mg/kg, as this was the only dose that significantly impaired 'no-go' accuracy in an analysis of data from a subgroup of the final *n*, who were tested earlier with a greater range of doses (fig. 4).
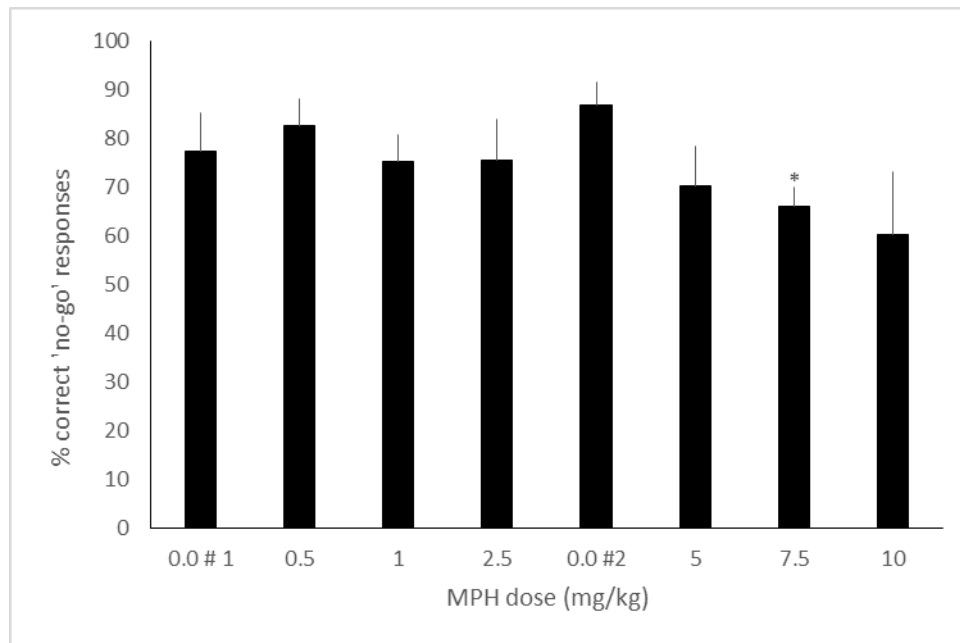
*Significantly decreased from baseline 0.0 #2, p < .0125.

Figure 4. Mean percentage accuracy in the no-go trials (± SEM) for the subgroup of rats (n=9) that began the experiment first. A range of MPH doses were tested on these rats, only some of which were tested on the other four rats that made up the final group and further analyses. Repeated measures ANOVA run on the no-go response data showed a significant effect of MPH dose on 'no-go' accuracy, $F(2.28, 18.26) = 3.77$, *p*< .05. Multiple paired-samples t-tests with a Bonferroni corrected alpha value of p < .013 were run between saline(0.0 mg/kg) #1 and the 1.0 mg/kg dose, and saline#2 and each of the three highest doses. The 0.5 mg/kg and 2.5 mg/kg doses were not included as it was necessary to include them in the final analysis regardless, for comparisons with later treatments relating to specific hypotheses about that dose. Of the four doses tested, the only treatment to significantly alter baseline behaviour was the 7.5 mg/kg dose (65.92 ± 3.95), which decreased no-go accuracy compared with saline #2 (M = 86.75, SD = 14.147), t(8) = 7.76, p < .013.

A mixed ANOVA found no main effect of lever latency F(2, 22) = 2.27, p = .127, indicating that the latency of the 'no-go' signal did not affect overall 'no-go' accuracy. There was a main between-subjects effect of impulsivity F(1, 11) = 8.44, p < .05, confirming that the median split based on overall accuracy in no-go trials had separated the animals into two significantly different groups, the HI group being significantly less accurate.

None of the interactions between the IV's were significant, indicating that a) both the HI and LI groups displayed a similar response pattern to the increasing doses of MPH, and b) the latency of the 'no-go' signal did not affect accuracy differently depending on whether the animals were HI or LI, or what dose of MPH they had taken. Therefore further analyses of no-go trials included the whole group of rats, and averages of all latencies combined.

3.2.1.2. Effect of MPH dose on no-go accuracy

In addition to the saline treatment included as baseline in the above analysis, saline #2 was included as baseline comparison for the 7.5 mg/kg dose of MPH. Counter to the hypothesis that the 0.5 mg/kg dose would improve 'no-go' accuracy, there was no main effect of MPH on 'no-go' responding, $F(4, 48) = 1.83$, $p = .180$ (fig. 5). The 7.5 mg/kg dose did yield lower 'no-go accuracy' compared to its baseline, saline #2, it was not enough to contribute to a significant main effect, unlike the 7.5 mg/kg dose in the original group of 9.
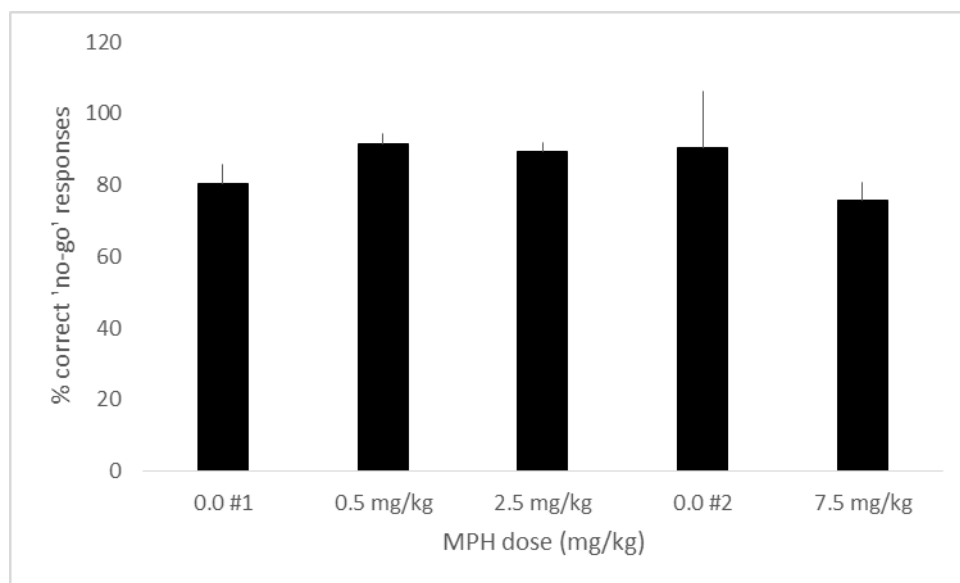
Figure 5. Group means (n=13) for percentage accuracy in the no-go trials (± SEM) across different doses of MPH (0.0 mg/kg treatments were saline injections). Repeated measures ANOVA did not find an effect of MPH on no-go accuracy, $F(4, 48) = 1.83$, $p = .180$.

3.2.2. 'Go' accuracy

The other dependent variable that was measured was 'go' accuracy. This provided a measure of general arousal and/or attention, alongside changes in 'no-go' accuracy. A decrease in 'go' accuracy coupled with decreased 'no-go' accuracy could indicate a drop in attention, whereas an increase in go accuracy paired with no change, or a decrease in 'no-go' accuracy could point to generally increased motor arousal.

3.2.2.1. Influence of lever latency and rats' individual baseline impulsivity on the effects of MPH on 'go' accuracy.

In order to determine if MPH influenced go accuracy, but only for certain lever latencies or for rats that were particularly high or low impulsivity, a mixed ANOVA identical to that performed on the 'no-go' data was run. A main effect of lever latency was found, $F_{(2, 22)} = 48.51$, $p < .05$. The shorter lever latency trials did appear to be more difficult for the rats to make a 'go' response in, with post hoc tests showing rats made more errors (no response) during go trials utilising the shortest latency, 3 s, than the two longer ones, with both 6 s and 9 s trials yielding more accurate go responding overall (fig. 6). However the lack of an interaction with MPH dose shows that latency is affecting 'no-go' accuracy similarly across all doses, including baseline. This indicates that there is no need to look at effects of MPH for each trial type separately.
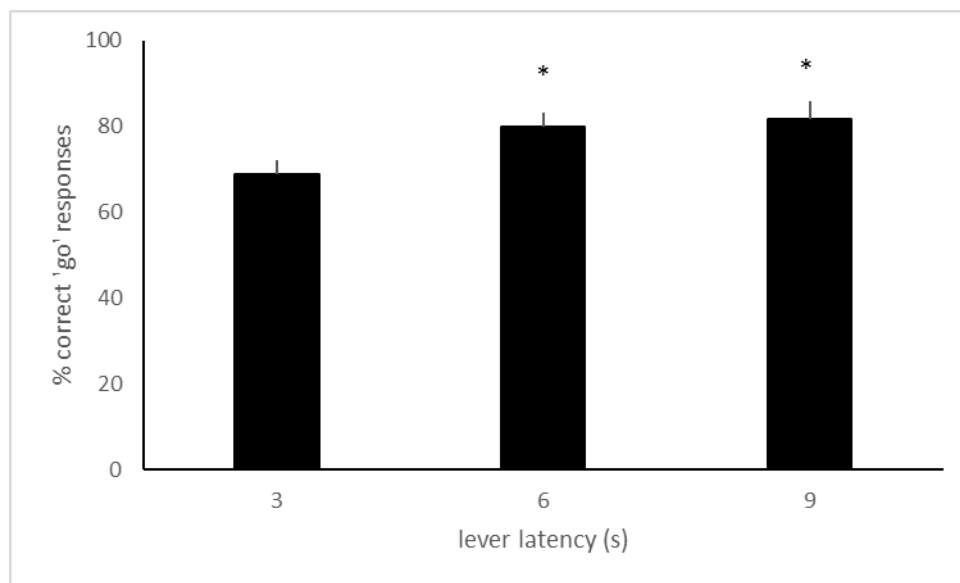
*Significantly increased from 3 s latency condition, $p < .05$

Figure 6. Group means (n=13), with MPH doses and baselines combined, for overall percentage accuracy in the 'go' trials (± SEM) across the three different trial types (latencies). Post-hoc contrasts demonstrated an overall increase in go accuracy in the 6 s condition (82.39 ± 3.41) compared with the 3 s condition (74.45 ± 3.27), $p < .05$, and also in the 9 s condition (84.45 ± 3.93) compared with 3 s, $p < .05$.

Rats' baseline impulsivity level had no effect on 'go' accuracy, which indicates that the measurement of impulsivity (accuracy of responding in no-go trials) does not seem to be related to accuracy of 'go' responding. It was possible that rats that could potentially have been classified as 'less impulsive' not because they correctly withheld more responses during

'no-go' trials, but because of a low overall response rate, which would be indicated by a lower accuracy in go trials. However, this finding provides evidence that the measures are independent.

To further verify this view, the 'go' and 'no-go' responding scores for averaged latencies in all drug treatment conditions (resulting in 129 paired cases) were correlated using Pearson's correlation, which found no significant relationship between the two variables, $r = -.107$, n= 129, $p = .228$. Thus, a lower 'go' trial accuracy did not lead to higher 'no-go' trial accuracy, giving us confidence that the changes in 'no-go' scores truly reflect changes in impulsivity rather than a general reduction in responding.

As no significant interactions were observed between MPH dose and lever latency or baseline impulsivity, it was acceptable to analyse the effects of MPH dose for all rats in the experiment and all lever latencies combined.

3.2.2.2. Effect of MPH dose on go accuracy

A main effect of MPH was found on accuracy in the go trials, with saline #2 included in this analysis as a baseline comparison for the 7.5 mg/kg dose of MPH. Selected post-hoc t-tests compared each dose with its baseline. These tests indicated that that go accuracy was significantly compromised by the 7.5 mg/kg dose of MPH compared to its baseline, saline #2 (fig. 7). This was the only dose that affected go accuracy. Paired with the drop (significant in the original subset of 9 rats, but not in the whole group) in no-go accuracy produced by the 7.5 mg/kg dose of MPH (see above), it can be postulated that MPH at this dose leads to a significant lowering of attention in rats.
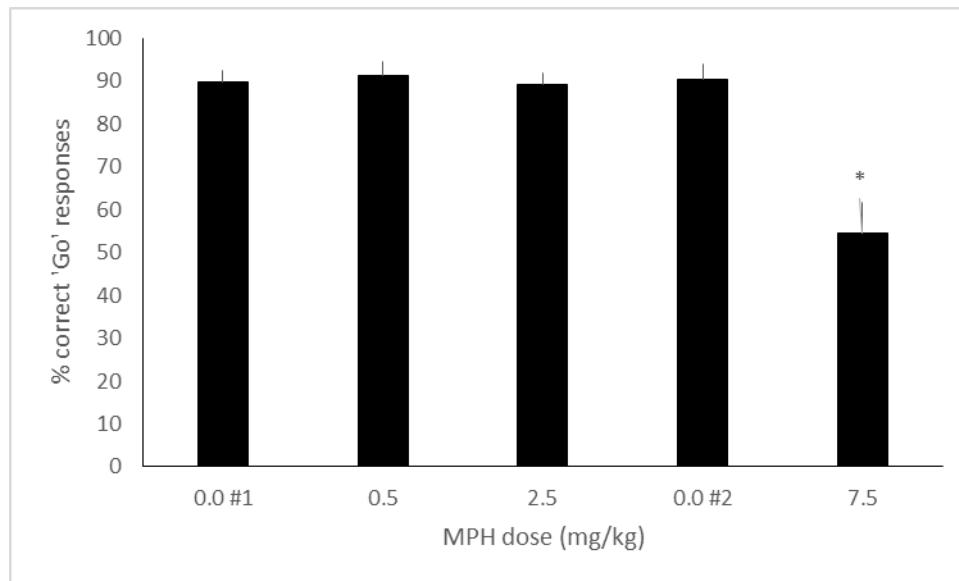
*Significantly decreased from baseline 0.0 #2, p < .0167

Figure 7. Group means (n=13) for percentage accuracy in the go trials (± SEM) across different doses of MPH. A main effect of dose was found, F(1.42, 17.01) = 19.5, p < .05. Post-hoc t-tests were performed between each dose and its baseline-0.5 and 2.5 mg/kg with saline #1 and 7.5 mg/kg with saline #2. The source of the effect was a significant drop in go accuracy at the 7.5 mg/kg MPH dose (54.51 ± 7.1) compared to its baseline, saline #2 (90.49 ± 3.4), t(12) = 4.93, p < .0167.

3.3. Effects of FLX alone

3.3.1. 'No-Go' accuracy

FLX administered at doses of 1.0 mg/kg and 5.0 mg/kg did not have an effect on 'no-go' accuracy F(2, 20) = .10, p = .91, as demonstrated by a mixed ANOVA comparing these two doses and their baseline, saline #3. There was also no effect of lever latency, F(2, 20) = .21, p = .81. A main effect of baseline impulsivity F(1, 10) = 5.05, p < .05 was found, with HI rats (M = 68.87, SE = 6.9) showing lower overall 'no-go' accuracy than LI rats (M = 90.81, SE = 6.9), however no interactions were found between any of the independent variables, allowing us to conclude that FLX did not have an effect on 'no-go' accuracy for any of the trial types or impulsivity levels.

3.3.2. 'Go' accuracy

When accuracy in the 'go' trials was examined with a mixed ANOVA, comparing the same three treatments as the above section, FLX did not have an effect on 'go' accuracy F(1.21, 12.12) = 1.91, p = .19, and no effect of baseline impulsivity level was found, F(1, 10)

= .03, p = .87. Latency significantly affected overall 'go' accuracy, with post-hoc t-tests between latencies (all doses included) showing a similar effect to that which was observed on 'go' accuracy during the MPH treatments, with the 3 s latency conditions proving the most 'difficult'. Rats were less accurate in the 3 s latency condition (M = 86.38, SD = 14.79) than the 6 s condition (M = 92.63, SD = 10.68), t(35) = -4.78, p < .001, but accuracy in the 9 s condition (M = 91.11, SD = 17.05) did not differ from either of the others.

3.4. Effects of the combination of MPH and FLX compared to each drug administered alone.

3.4.1. 'No-go' accuracy

Since none of the comparisons in the following analyses involved saline #2, the average of saline #1 (baseline for the 0.5 mg/kg and 2.5 mg/kg doses of MPH) and saline #3 (baseline for the 5.0 mg/kg and 1.0 mg/kg doses of FLX, and both of the combined FLX/MPH treatments), which were extremely similar (see fig. 3), was used as the baseline for all treatments.

Neither 0.5 mg/kg MPH (M = 84.49, SD = 14.35), t(12) = -1.3, p = .22 nor 5.0 mg/kg FLX (M = 84.63, SD = 15.71), t(12) = -1.29, p= .22 alone had an effect on baseline no-go accuracy (M = 80.19, SD = 20.34), nor was there an effect when they were administered in combination (M = 84.89, SD = 14.45) t(12) = -1.54, p = .15. Hence, unsurprisingly, when directly comparing the MPH treatment to that when it was combined with FLX, no difference could be detected, t(12) = .143, p = .89, thus no effect of FLX on the 0.5 mg/kg dose of MPH can be claimed.

Similarly, the higher MPH dose of 2.5 mg/kg made no difference to baseline 'no-go' accuracy, (M = 81.43, SD = 13.95), t(12) = -.20, p = .845, and the co-administration of 5.0 mg.kg FLX alongside this dose also did not affect baseline (M = 81.54, SD = 22.61), t(12) = .331, p = .747.

3.4.2. 'Go' accuracy

The two relevant baseline phases, saline #1(M = 89.63, SD = 9.76) and saline #3 (M = 93.82, SD = 10.21), were not significantly different, t(12) = -1.637, p = .128, so the data from each was averaged as in the 'no-go' analysis.

A repeated measures ANOVA comparing go performance under the averaged saline, the 0.5 mg/kg MPH, the 5.0 mg/kg FLX, and the 0.5 MPH + 5.0 FLX conditions did not find a main effect of treatment, F(3, 36) = .69, p = .57.

The effects of the 2.5 mg/kg dose of MPH however were significantly altered by the co-administration of 5.0 mg/kg FLX (fig. 8). An ANOVA comparing go accuracy across the average saline, 2.5 mg/kg MPH, 2.5 mg/kg MPH + 5.0 mg/kg FLX, and 5.0 mg/kg FLX conditions found a main effect of treatment. The source of this was a drop in 'go' accuracy from baseline when 2.5 mg/kg MPH was administered in combination with 5.0 mg/kg FLX. Neither MPH nor FLX administered alone, at these doses, affected baseline 'go' responding, indicating that it was a synergistic effect of the combination of the two that decreased 'go' accuracy.
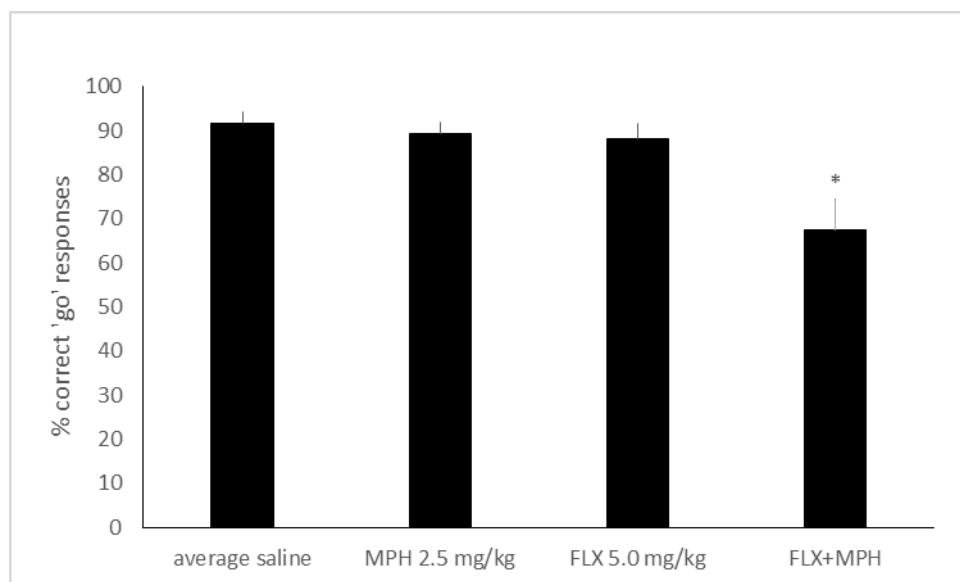
*Significant decrease from averaged saline (baseline), p < .01

Figure 8. Group means (n=13) for percentage accuracy in the 'go' trials' (± SEM) across different drug treatments. ANOVA found a main effect of treatment, F(1.52, 18.22) = 6.82, p < .05 for the 'go' trials. Post hoc t-tests showed that MPH alone (M = 89.25, SD = 9.3) did not affect baseline go accuracy (M = 91.72, SD = 8.86) t(12) = -1.23, p = .241, but the combination of the two drugs (M = 67.49, SD = 25.35) was followed by a significantdecrease, t(12) = 3.13, p < .01. This effect of co-administration could not have been induced by the FLX, as 5.0 mg/kg FLX administered alone (M = 88.27, SD = 11.7) did not alter baseline go accuracy, t(12) = -.86, p = .405

3.4.3. Possibility of attentional effects

The lack of an increase in 'no-go' accuracy for the 2.5 mg/kg MPH + 5.0 mg/kg FLX treatment suggests that an overall lowering of attention may be held to account for the decrease in 'go' responding, rather than a broad reduction in responding overall. Animals were responding less in the 'go' condition, resulting in a lower 'go' accuracy score, but they were not responding less in the 'no-go' condition, which would increase 'no-go' accuracy. However if the rats' overall attention was lowered it could be expected that their 'no-go' accuracy would decrease (their response rate would increase).

To investigate the causes of the drop in go accuracy further, a Pearson's correlation was conducted between 'go' and 'no-go' accuracy for the 2.5 mg/kg MPH + 5.0 mg/kg FLX treatment only. As with the correlation performed across all treatments (see above), there was not a significant association between 'go' responding and 'no-go' responding, $r = -.429$, $n = 13$, $p = .143$. This shows that the animals with lower 'go' accuracy in this condition did not tend to have higher 'no-go' accuracy, which supports the argument that a reduction in attention is the cause of the drop in 'go' accuracy.

4. Experiment 2: SSRT results

The purpose of this experiment was to examine the effect of a range of MPH and FLX doses on stopping latency, a measure of 'action cancellation' type impulsivity. It was predicted that lower doses of MPH would decrease stopping latency, an indication of decreased impulsivity. FLX was not predicted to have an effect by itself, but to perhaps potentiate MPH's impulsivity-decreasing effects when administered in combination with it.

4.1. Multiple baselines

Although the animals were trained to a 'stable' level before testing began, a practice effect was still apparent. A paired samples t-test between average scores for the first block of saline sessions (M = 440.44, SD = 65.74) and a second saline session conducted 81 days after the first block (M = 404.89, SD = 54.63) revealed a significant group reduction in stopping latencies $t(12) = 3.402$, $p < 0.05$, probably due to further practice with the task.

For this reason the first saline session (saline 1) is used as the baseline comparison for MPH doses 0.5, 1.0 and 2.5 mg/kg. The second saline session (saline 2) is included in the analyses as the baseline comparison for MPH doses 5 and 7.5 mg/kg, both of the FLX doses, and all of the treatments where FLX and MPH were co-administered, as the placement of these treatments on the timeline of the experiment falls closer to the second saline session than the first.

4.2. Trial difficulty as determined by number of fixed responses (FR) required before stop signal appeared.

As mentioned in the method section, trials varied in how many lever presses were required before either a) reward was delivered (in go trials) or b) stop signal was presented (in stop trials). The stop trials operated under one of three FR schedules- FR2, FR5 and FR8, to vary the difficulty level of stopping the sequence of responding. The FR8 trials were intended to be the most difficult while the FR2 trials were intended to be easiest, however the opposite was revealed to be true, with the more lever presses made before the appearance of the stop signal, the faster the stopping latency (fig. 9). For all further analyses, the FR factor shall be referred to as 'trial difficulty', with the FR8 trials being referred to as 'easy' and FR2 trials being referred to as 'difficult'. FR 5 trials will not be included as a level of this factor.
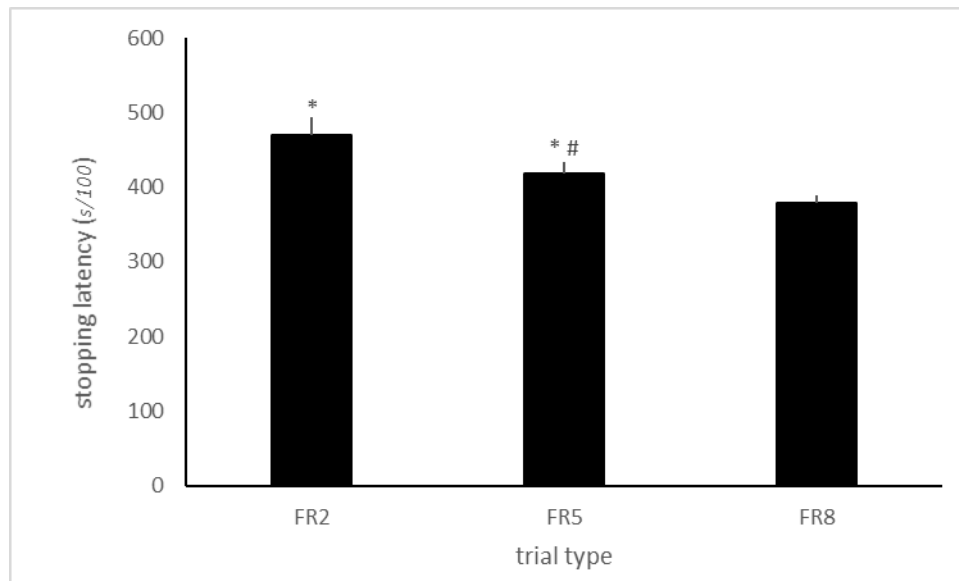
*Significant increase from FR8 trials, p < .05

#significant decrease from FR2 trials, p < .05

Figure 9. Mean baseline (saline #1) stopping latency ($\pm$ SEM) for the whole group of rats (n = 13), across the three different FR trial types. A repeated measures ANOVA found a significant effect of trial type on stop latency, $F(2, 24) = 36.14$, p < .05. Post-hoc t-tests showed stopping latencies in the FR8 trials (M = 379.21, SD = 38.03) to be significantly faster than in both the FR5 trials (M = 418.45, SD = 57.23), $t(12) = 6.25$, p < .05, and FR2 trials (M = 470.34, SD = 81.71), $t(12) = 6.45$, p < .05, and FR5 stopping latencies being significantly reduced compared to FR2, $t(12) = 5.0$, p < .05.

The reason behind this unexpected finding may be that the rats were responding at a slower rate by the time they got to the last few presses in the trials with high FR, due to an expectation that the stop signal may be coming soon. This is in contrast to the short FR trials in which the stop signal appeared after the first few presses, at which stage the number of required FR's is still ambiguous to the rat. However, although there was a trend for slower response rates the higher the FR, there was no significant difference in response rates between the trial types $F(2, 24) = .95$, $p = .40$, making this theory difficult to prove.

4.3. Effects of MPH on stopping latency

The HI group (M = 434.07, SD = 89.84) were significantly slower at stopping than the LI group (M = 390.15, SD = 54.49), $F(1, 11) = 7.48$, p > .05, confirming that the median split had successfully differentiated two groups of rats in terms of their stopping latency. An interaction between baseline impulsivity and dose, $F(2.55, 27.99) = 7.11$, p > .05 indicated that the effect of MPH dose on stopping latency was different depending on the baseline stopping speed of the rat (fig. 10).

Further analyses revealed that MPH had no effect on stopping latency for the LI group, possibly due to a ceiling effect, as their baseline stopping speeds were already fast. However the HI group's stopping latencies were significantly affected by the MPH treatment. When comparing each dose with its baseline, it was found that the 1.0 and 2.5 mg/kg doses of MPH reduced stopping latency compared to their baseline, saline #1, while there was a trend for the 7.5 mg/kg dose to as well but this did not reach significance.

Trial difficulty did not interact with MPH dose for any of the comparisons of interest, those being each dose compared with its baseline.
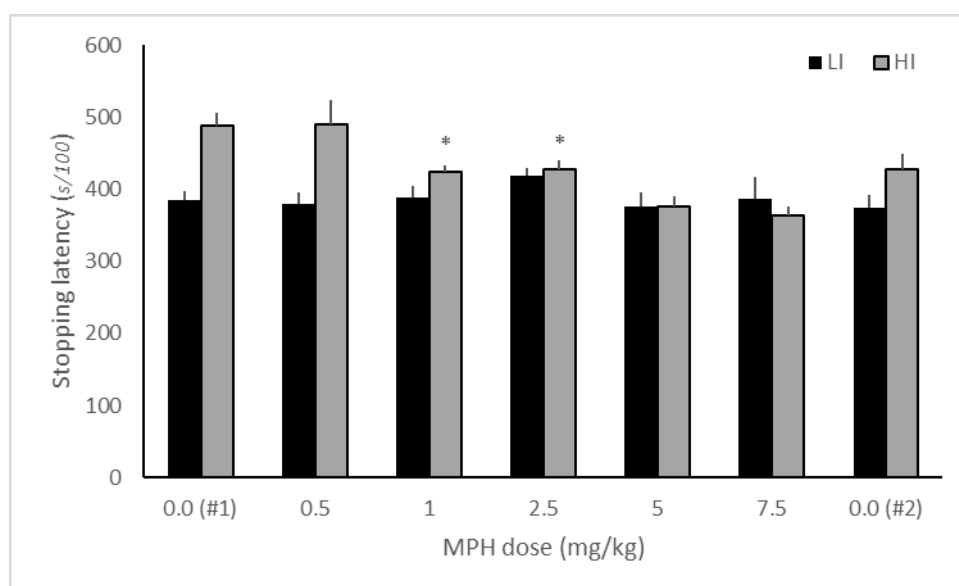
*significant decrease from saline #1, p < .01

Figure 10. Mean stopping latency (± SEM) across MPH doses and baselines, for the LI and HI groups. ANOVA for LI group (n = 6) showed no overall effect of dose stopping latency $F_{(6, 30)} = 1.15$, p = .358. ANOVA for HI group only (n = 7) revealed a significant main effect of dose, $F_{(6, 36)} = 9.24$, p < .05. Paired t-tests were performed on each dose with its baseline for the HI group. Significant differences in stop latency was found between saline #1 (M = 488.29, SD = 44.78) and 1.0 mg/kg MPH (M = 424.88, SD = 22.67), $t_{(6)} = 4.69$, p < 0.01, and also between saline #1 and 2.5 mg/kg MPH (M = 427.02, SD = 32.59), $t_{(6)} = 3.87$, p < 0.01. Both of the higher doses of 5.0 mg/kg (M= 376.61, SD = 37.78) and 7.5 mg/kg MPH (M = 363.59, SD = 34.46) did not make a significant difference to stop latencies measured during the baseline sessions relevant to them (saline2, M= 430.96, SD = 53.37), although the 7.5 mg/kg dose approached significance $t_{(6)} = 3.15$, p = 0.02.

To investigate whether change in the rate of responding was contributing to the observed decreases in stop latency in the 1.0 and 2.5 mg/kg conditions, another ANOVA was conducted across MPH doses, using data from the HI group and the FR2 trials only (as these are where stopping latencies are longest, giving the most extra responses to calculate rate

from) and measuring response rate as the DV. However this analysis found no main effect of MPH dose on rate of responding, $F(6, 36) = 1.26$, $p = .298$.

4.4. Effects of FLX on stopping latency

A main effect of dose was found for the FLX treatments $F(2, 22) = 4.35$, $p < .05$, as well as a main effect of trial difficulty, $F(1, 11) = 59.5$, $p < .05$, and baseline latency $F(1, 11) = 5.27$, $p < .05$. There was a significant interaction between the factors dose and trial difficulty, with no treatment effects occurring within the easy trials, but a significant effect of FLX dose within the difficult trials (fig. 11). The source of this variation was a significant reduction in stopping latency in the 1 mg/kg FLX condition compared to both the 5 mg/kg FLX condition, and baseline.
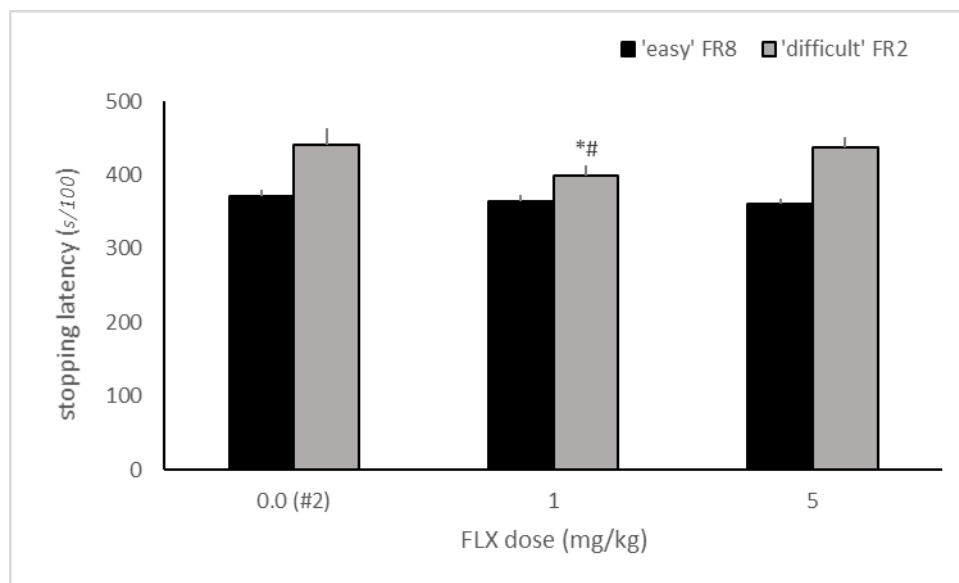
*significant decrease from baseline (0.0 #2) $p < .017$

#significant decrease from 5 mg/kg FLX treatment $p < .017$

Figure 11. Mean stopping latency ($\pm$ SEM) across FLX doses and baseline. An ANOVA performed on the easy FR8 trials did not find a main effect of FLX dose, $F(2, 24) = 1.18$, $p = 0.325$. The ANOVA analysing the difficult trials however did find a main effect of dose, $F(2, 24) = 5.19$, $p < .05$. Post-hoc t-tests for this group found a significant reduction in stopping latencies in the 1.0 mg/kg FLX condition ($M = 399.82$, $SD = 47.14$) compared to baseline ($M = 441.59$, $SD = 80.29$), $t(12) = 3.11$, $p < .017$, and the 5.0 mg/kg FLX condition ($M = 437.28$, $SD = 48.09$), $t(12) = -3.48$, $p < .017$.

The significant effect of 1.0 mg/kg FLX could have been due to a general slowing down of responding. If animals are pressing the lever at a slower rate, they will be more

likely to make no response within the 3 second time window that constitutes a successful 'stop'. Potentially, it may be that pressing slower also makes it generally easier to stop.

However an ANOVA, performed on data from the FR2 trials only, found no main effect of FLX on rate of responding, $F(2, 24) = .24$, $p = .68$, making the slowing of the response rate an unlikely contributor to the effect of FLX on stopping latency.

4.5. Effects of the combination of MPH and FLX compared to each drug administered alone.

As there appeared to be a ceiling effect for the LI group, with no significant decreases in stopping latency being observed with the various doses of MPH administered, the following analyses were only made for the HI group.

4.5.1. 0.5 mg/kg MPH alone compared to co-administration with 5.0 mg/kg FLX.

As described above, the lowest, 0.5 mg/kg dose of MPH did not significantly affect baseline (saline 1) stopping latency. Even though the 0.5 mg/kg MPH dose failed to alter baseline scores, this dose was administered with 5.0 mg/kg FLX to investigate whether a speeding of stopping latencies could be observed. T-tests showed that while the 0.5 mg/kg MPH dose did not affect baseline stopping speeds $t(6) = .042$, $p = .97$, when it was administered with 5.0 mg FLX it tended to result in a lower mean stopping latency compared to this treatment's baseline, saline #2 (although this difference did not reach significance ($t6) = -1.08$, $p = .322$). This non-significant 'improvement' in the combined condition however may be due to actions of the FLX alone, rather than a synergistic effect, as 5.0 mg/kg FLX also tended to yield faster mean stopping speeds than saline 2 sessions, although again this did not reach significance, $t(6) = 1.64$, $p = .15$ (fig. 12).
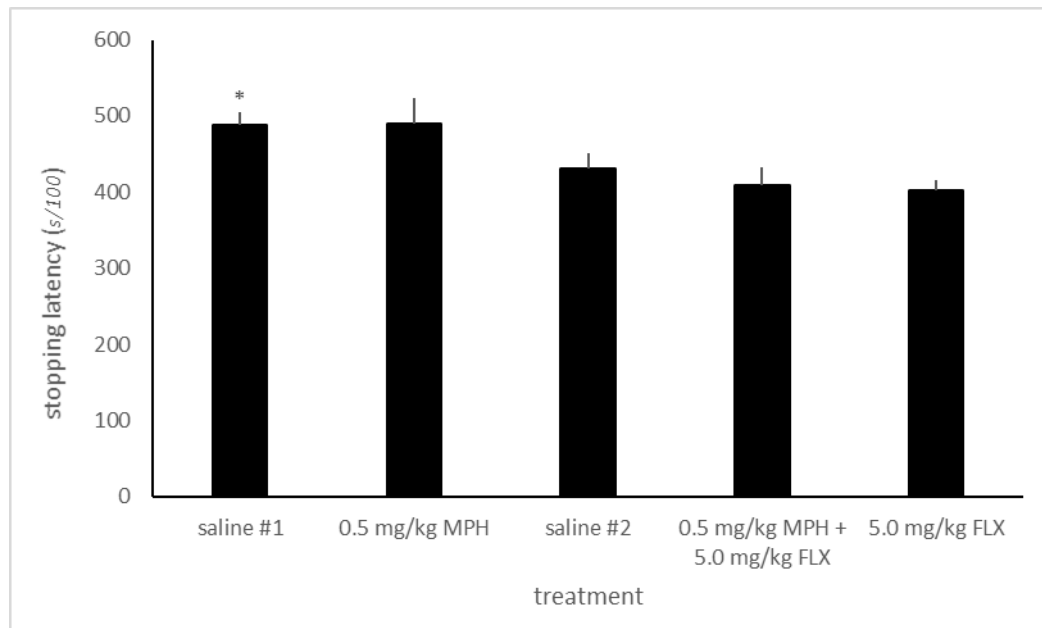
*Significantly higher than saline #2, p < .05

Figure 12. Mean stopping latency (± SEM) of HI group (n = 7) across different drug treatments. T-tests showed the 0.5 mg/kg MPH dose (M = 489.14, SD = 91.09) to cause no significant change to baseline (saline 1, M = 488.29, SD = 44.78) stopping speeds t(6) = .042, p = .97. The 0.5 mg/kg dose of MPH, in combination with 5.0 mg FLX, resulted in a lower mean stopping latency (M = 408.89, SD = 62.79) compared to its baseline, saline2 (M = 430.96, SD = 53.37) although this difference did not reach significance (t6) = -1.08, p = .322. FLX administered alone had a similar effect to the combination treatment, yielding a faster mean stopping speed (M = 402.42, SD = 34.59) than saline 2 sessions, again not reaching significance, t(6) = 1.64, p = .15.

4.5.2. 1.0 mg/kg MPH alone compared to co-administration with 5.0 mg/kg FLX.

As 1.0 mg/ kg MPH can be considered a clinically relevant dose, it is pertinent to investigate if there is any effect of FLX on this dose of MPH.

Unlike 1.0 mg/kg MPH alone, which reduced stopping latencies, the administration of 5.0 mg/kg FLX at the same time as 1.0 mg/kg MPH did not alter baseline stopping latencies. Howeverthe practice effect, which significantly improved baseline scores between saline 1 and saline 2, makes it possible that a ceiling effect may be present in the combination treatment, with already fast baseline stopping latencies being resistant to improvement. The difference in baseline performance also negates any comparison between the MPH and combined treatments, which is needed if we are to show any attenuation of the latency reducing effects of MPH by FLX. To address this issue, the 1.0 mg/kg MPH and the 1.0 mg/kg MPH + FLX treatments were compared directly using the percentage change from baseline as the DV, again using only the HI group's data. No difference was found between

the MPH and combined treatments, which implies FLX did not have an attenuating effect on the improvement in stopping speed induced by 1.0 mg/kg MPH.

4.5.3. 2.5 mg/kg MPH alone compared to co-administration with 5.0 mg/kg FLX.

2.5 mg/kg MPH significantly reduced baseline stopping latencies for the HI group, $t(6) = 3.87$, $p < .05$. However this effect was abolished when this MPH dose was administered alongside 5.0 mg/kg FLX. The lack of a significant effect of the combination treatment indicates that it is possible that the FLX is attenuating the effect of the MPH to some degree. It is also possible that the lack of a significant effect of the 2.5 mg/kg MPH + 5 mg/kg FLX treatment is due to the overall improvement of baseline scores, introducing a ceiling effect.

To elucidate this matter, further 2.5 mg/kg MPH only sessions were conducted later in the timeline, which were able to share saline 2 as a baseline with the combined treatment. The later 2.5 mg/kg MPH treatment did not alter baseline stopping latency, $t(6) = 1.175$, $p = .284$, nor did it differ from the combined treatment, $t(6) = -.131$, $p = .90$.

This suggests that a practice-induced ceiling effect may have been responsible for the discrepancy between the stop-latency speeding effects of MPH alone, and the absence of this effect when administered alongside FLX, when the 2.5 MPH data from the original, earlier time-point was used.

To attempt to overcome this issue, the original 2.5 mg/kg MPH treatment was once again compared to the 2.5 mg/kg MPH + 5.0 mg/kg FLX treatment, again with the slow group only, but this time the stopping latencies' percentage change from baseline under the different treatments was used as the DV, allowing treatments to be compared directly rather than via their differences from separate baselines. However this type of analysis also failed to find a difference in stopping latencies between 2.5 mg/kg MPH administered alone, or with 5.0 mg/kg FLX, $t(6) = -.456$, $p = .664$.

5. Experiment 3: Locomotor activity results

This experiment was conducted to assess the effects of several doses of MPH on baseline motor activation and compare them to their effects when they were administered in

combination with 5.0 mg/kg FLX. It was expected that FLX would would potentiate the locomotor activity-enhancing effects of MPH, whilst not influencing activity when administered alone.

5.1. Effect of treatments over time

Drug effects can vary over a 70 minute time-course. To determine whether differences in activity levels (measured by number of ambulatory counts) between the six treatments were more pronounced during certain time-periods within the 70 minutes that activity was recorded, a time x treatment repeated-measures ANOVA was performed. The results of this analysis were used to determine whether it was necessary to compare treatment effects on ambulatory counts across subsequent smaller blocks of time, or for the whole 70 minutes combined.

Instead of having every five-minute time bin as a separate level of the time factor, the first six bins were entered as separate levels, and the seventh level comprised of the sum total of ambulatory counts over the following eight time bins, as the time series graph (fig. 14, p. 75) suggested that strongest effects of treatment were occurring in the first 30 minutes of recording. The ANOVA showed a main effect of treatment, $F(2.12, 50.82) = 7.6$, $p < .001$

The ANOVA revealed a time x treatment interaction, $F(30, 720) = 1.78$, $p < .01$ indicating that the effects of treatment type on activity may have varied over the time course of activity recording. One-way ANOVA were performed on treatments for each of the first six time bins and the amalgamated 'seventh' time-bin (comprised of the last eight time-bins) separately. The first six time bins showed a significant treatment effect, however the amalgamated 'seventh' time bin did not show an effect. Thus, effects of treatments on ambulatory counts were only compared for the period covering the first six time bins (minutes 5-35), as this is clearly when the treatments were having their strongest effect, before tailing off.

5.2. Comparison between drug treatment effects (5-35 minutes post injection(s))

Total ambulatory counts recorded between minutes $5 - 35$ post treatment were significantly influenced by drug treatments, $F(1.6, 36.9) = 14.5$, $p < .05$.Selected post-hoc t-

tests were performed on comparisons of interest, with a Bonferroni-corrected alpha level of p < .006. Neither of the MPH doses (0.5 and 2.5 mg/kg) or the FLX (5.0 mg/kg) had a significant effect on activity compared to saline baseline. Combining the FLX dose with the 0.5 mg/kg dose of MPH did not influence activity either. However, administering 5.0 mg/kg FLX in combination with the 2.5 mg/kg dose of MPH resulted in a significant rise in activity compared with either drug administered alone, as well as saline baseline (fig. 13). The activity-lowering effect of FLX administered alone, while not significant, makes it unlikely that the increase in activity is an additive effect of the two drugs.
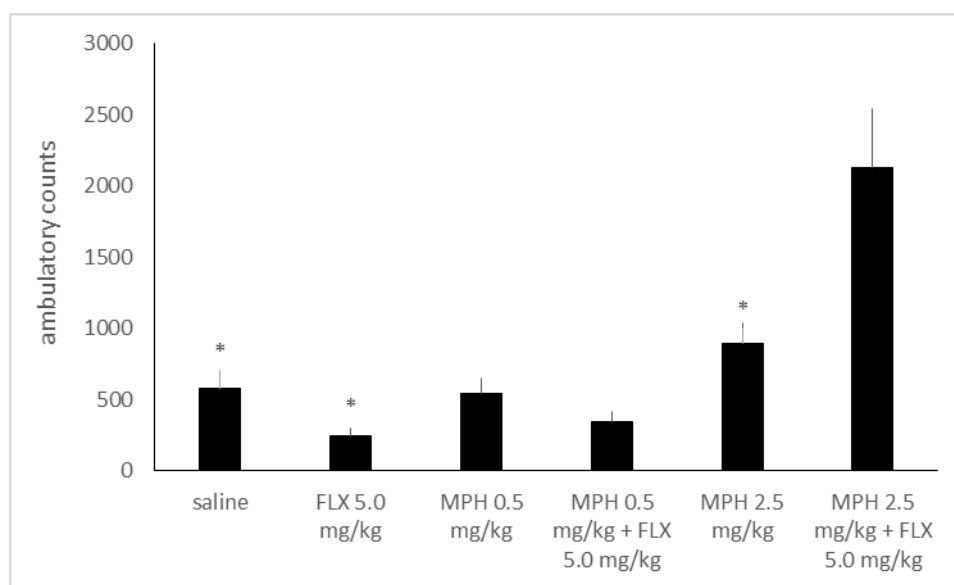
*significantly lower number of ambulatory counts than the combined 2.5 mg/kg MPH + 5.0 mg/kg FLX condition.

Figure 13. Group mean (n = 25) number of ambulatory counts (± SEM) for each of the six treatments. A one way repeated-measures ANOVA showed a significant treatment effect, $F(1.6, 38.39) = 14.17$, $p < .05$ (.001). Post –hoc t-tests revealed the number of ambulatory counts made between minutes 5-35 post-injection(s) to be significantly higher in the MPH 2.5 mg/k + FLX 5.0 mg/kg (M = 2129.8, SD = 2080.1) condition than in the saline control condition (M = 575.9, SD = 578.5), $t(24) = -3.45$, $p < .006$. They also showed counts in this combined condition to be higher than in the conditions where each of the drug doses were given alone, MPH 2.5 mg/kg (M = 889.76, SD = 736.22), $t(24) = -3.25$, $p < .006$, and FLX 5.0 (M = 243.88, SD = 293.37), $t(24) = -4.68$, $p < .006$. None of the other comparisons between treatments that were tested reached significance.

5.3. Associations between effects of drug treatments on activity and on impulsivity in the Go/No-Go and SSRT tasks.

Although there was not an overall significant improvement in 'no-go' accuracy seen in the 0.5 mg/kg MPH condition in the Go/No-Go task, there was a trend towards improvement. Some of the rats showed a great deal of improvement while others did not. In order to determine if there was a relationship between the degree of change in activity

produced by 0.5 mg/kg MPH and the extent to which 'no-go' accuracy was improved by that same treatment, a two-tailed Pearson's correlation was performed using data from the 0.5 mg/kg MPH condition from the 12 rats which performed in both the Go/No-Go task and the locomotor activity monitoring. The variables used were the change from baseline for ambulatory counts, and the 'no-go' accuracy score's change from baseline. Percentage change was not used as two of the rats' baselines for locomotor activity were zero, which skewed the data. The correlation did not show a relationship between change in activity and change in 'no-go' accuracy, $r = .28$, $n = 12$, $p = .38$.

The within-subjects variation in activity levels in the 2.5 mg/kg MPH + 5.0 mg/kg FLX condition of the locomotor experiment was substantial. To investigate whether any of this variation was associated with variation found in the same 12 rats in the Go/No-Go task, a second two-tailed Pearson's correlation was performed between locomotor activity change from baseline and 'no-go' accuracy change from baseline in the 2.5 MPH + 5.0 FLX treatment, relating to the hypothesis that higher DA release (indicated by increased activity) may increase impulsivity under this treatment condition in the Go/No-Go task. Again, this correlation was not significant. $r = -.084$, $n = 12$, $p = .80$.

A third two-tailed Pearson's correlation was performed to see if increases in ambulatory counts in the 2.5 MPH + 5.0 FLX condition were correlated with a drop in go accuracy in the Go/No-Go task (indicating a drop in attention). Again, the correlation showed no relationship between the two dependent variables, $r = -.337$, $n = 12$, $p = .29$.

5.4. Comparison between Sprague-Dawley and Wistar breeds

The locomotor task was the only task in which both breeds of rat were tested. There was a main effect of breed during the time period that was analysed (minutes 5-35), with Sprague-Dawleys (M = 484.54, SE = 148.16) moving less than Wistars (M = 1038.71, SE = 142.35) over all the treatments, $F(1, 23) = 7.28$, $p < .05$) However, breed did not interact with treatment, providing reassurance that the two breeds show relative similarity in their behavioural reaction to the different drug treatments, at least in terms of locomotor activity.
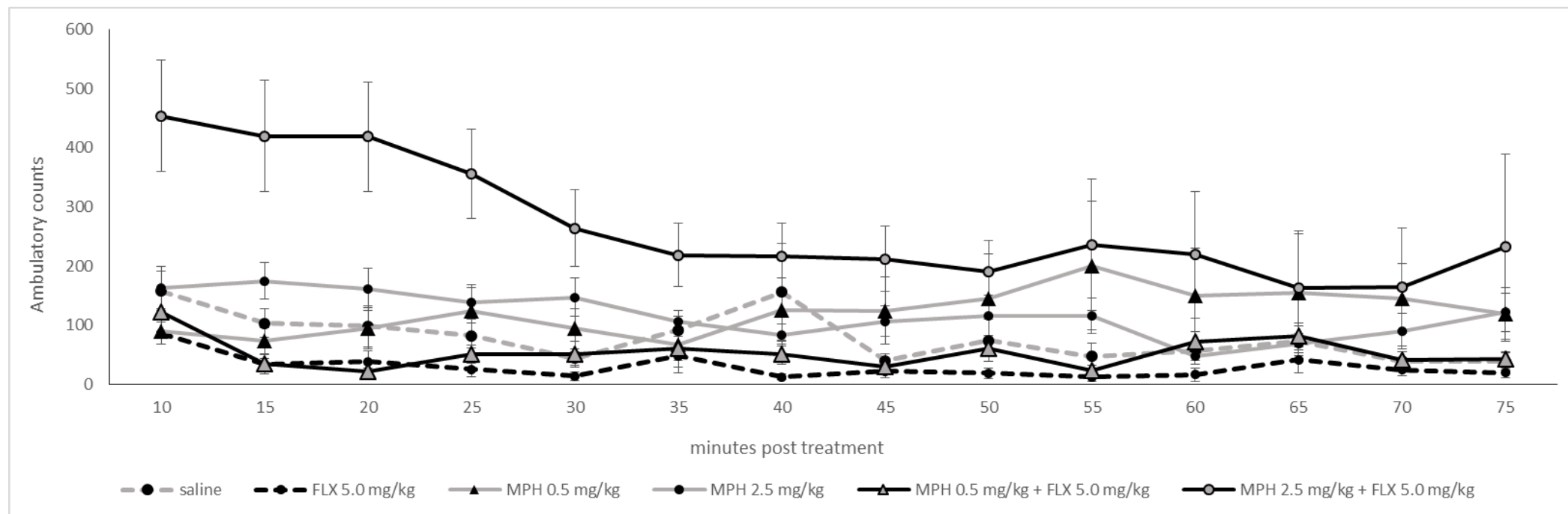
Figure 14. Group mean (n = 25) number of ambulatory counts (± SEM) for each of the six treatments over time. Ambulatory counts were recorded in five minute time bins, so each time-point reflects the mean sum of activity for the group of rats (n = 25) over the previous five minutes, starting from five minutes after drugs were administered. ANOVA found an interaction between time and treatment $F(2.12, 50.82) = 7.6$, $p < .001$, when the first 6 time bins were entered as separate levels of the time factor, and the last eight time bins were totalled as another level of the time factor.

6. Discussion

This study examined the effects of methylphenidate (MPH), a DAT/NET inhibitor commonly used in the treatment of ADHD, and fluoxetine (FLX), an SSRI used to treat depression, on two subtypes of impulsive behaviour – action restraint and action cancellation. The effects of the two drugs were investigated separately, then compared to the effects when administered in combination.

6.1. Go/No-Go Experiment

MPH did not have a significant effect on impulsivity ('no-go' accuracy) in the Go/No-Go task, however there was a trend for the 7.5 mg/kg dose to decrease this score, with a significant decrease present in a subgroup of the rats on which a larger variety of doses was piloted. As this was coupled with a significant decrease in 'go' accuracy at the 7.5 mg/kg dose, it can be inferred that the trend towards a decrease in 'no-go' accuracy is not indicative of increased impulsivity, but a decrease in attention. It is unlikely that the decrease in 'go' accuracy is due to a general slowing of responding or decrease in motor arousal, because the rats were in fact responding more frequently in the 'no-go' trials, leading to the trend towards a decrease in 'no-go' accuracy.

FLX did not affect either 'no-go', or 'go' accuracy in this task at either of the doses tested. The combination of 0.5 mg/kg of MPH and 5.0 mg/kg FLX did not alter 'no-go' or 'go' accuracy, however the combination of the slightly higher dose of MPH, 2.5 mg/kg, with the same dose of FLX (5.0 mg/kg) significantly reduced accuracy in the 'go' trials. These doses of MPH and FLX alone did not affect 'go' responding, thus the decrease can be attributed to a synergistic effect of the two drugs.

Again, the drop in 'go' accuracy with this treatment can most likely be considered an outcome of lowered attention rather than lowered arousal, as it was not accompanied by a corresponding increase in 'no-go' accuracy. It would be expected that if rats were generally responding more slowly overall, the percentage of successful 'no-go' trials would be pushed up- this was not the case however. Although a ceiling effect on mean 'no-go' accuracy could have prevented the scores from rising significantly higher, this is unlikely as the mean accuracy score for the combined treatment condition was not particularly high (81.4%) compared to the highest individual 'no-go' score obtained (97.9% in the baseline condition)

and was almost identical to baseline (M = 80.2). In addition to this, no association between 'go' and 'no-go' accuracy was found in the combined condition, showing that rats with lower go scores did not tend to have higher 'no-go' scores. The strongest argument against this drop in 'go' accuracy being a result of a decrease in motor arousal is that locomotor activity in the 2.5 mg/kg MPH + 5.0 mg/kg FLX condition was actually significantly increased relative to baseline, and the two drug doses administered alone.

Although a drop in attention should technically involve a decrease in accuracy in both 'go' and 'no-go' trials, the drop was only seen in the 'go' trials. This does not preclude an attention deficit as the reason behind deterioration in 'go' accuracy. A non-response on a 'no-go' trial could be interpreted as the rat paying attention and performing the correct behaviour for the context, or as the rat not attending to the light stimuli at all, and thus not *purposefully* withholding a response, but simply not paying attention to the task at hand. This is in contrast to the 'go' trials, for which a lever press in response to the light is a fairly unambiguous indicator that the rat was indeed paying attention to the task and the stimuli. This does make the observed behaviour difficult to pin definitively on an attentional effect of the drug combination however. An alternative explanation could be a decrease in motivation brought about by the synergistic effects of the two drugs. As with general arousal, we would expect to see a rise in 'no-go' accuracy if a decrease in motivation was to blame, but it is possible that 'no-go' scores were high enough to induce a ceiling effect and be insensitive to a drop in motivation.

This drop in 'go' responding, while not an initial focus of this study, is noteworthy. We expected to see behavioural effects of the combination of FLX and MPH in the form of an increase in action-restraint type impulsivity. The possible reasons that this was not observed will be discussed below, but first, the possible mechanisms behind either an attentional or motivational effect of the 2.5 mg/kg MPH + 5.0 mg/kg FLX combination will be explored.

Like impulsivity, attention can be monitored using the 5-CSRTT. It is measured by the percentage of unsuccessful trials that were not unsuccessful due to PR, but due to inaccurate nose-pokes being made (into the wrong hole) *after* the light turned on. All of the dopaminergic manipulations that were looked at in the context of impulsivity in the discussion can be re-examined to look at their effects on attention.

It does seem that an increase in extracellular DA is associated with a reduction in accuracy in the 5-CSRTT, with GBR12909 and AMPH leading to this effect (Baarendse & Vanderschuren, 2012; Harrison et al., 1997; Pattij et al., 2007; Van Gaalen et al., 2006), both systemically and when infused directly into the NAC. If increased DA release is responsible for the rats' drop in attention with the MPH/FLX drug combination, it could be due to activation at the D1 receptor, as D1 agonists have been show to decrease accuracy (Passetti et al., 2003a) and D1 antagonists can improve accuracy via systemic or intra-accumbal administration (Pattij et al., 2007; Van Gaalen et al., 2006). Conflictingly though, D1 agonists have also been demonstrated to increase accuracy in the 5-CSRTT when they are infused directly into the NAC or mPFC, and antagonists in these regions have had detrimental effects (Granon et al., 2000; Pezze et al., 2007), painting an inconsistent picture of the D1 receptor's role in visual attention. The other potential dopaminergic mechanism behind the drop in attention could be activation of the D2 receptors in the orbito-frontal cortex (OFC), as a D2 agonist infused into this region substantially impaired 5-CSRTT (Winstanley et al., 2010). The effect seems to be specific to this region, with systemic and intra-accumbal administration failing to influence accuracy (e.g. Moreno et al., 2013; Fernando et al., 2012; Pattij et al., 2007). Activation of OFC D2 receptors cannot be the sole mechanism though, as GBR12909 and AMPH still decreased accuracy when infused into the NAC. It is possible that accumbal D2 receptors do play a role in attention in conditions of abnormally high DA levels, as both systemic and intra-accumbal administration of a D2 antagonist attenuated the decrease in accuracy brought about by AMPH or mPFC lesioning (Passetti, Levita, & Robbins, 2003b; Pattij et al., 2007; Pezze et al., 2009), manipulations that increase DA efflux in the NAC (Dalley et al., 1999; Jackson & Moghaddam, 2001). Although it is difficult to draw any conclusions due to the inconsistencies in the research, based on these studies the activation of D2 receptors by the excess DA release in the PFC and NAC seems to be the most likely explanation behind a drop in attentional performance in the Go/No-Go task when rats were administered 2.5 mg/kg MPH + 5.0 mg/kg FLX.

As mentioned above, another factor that could be responsible for the drop in 'go' accuracy with the combined drug treatment is motivation. The rats may just be less motivated to perform the response that will deliver the sugar pellet, because they do not find the pellet as rewarding. There are several neurochemical explanations for this. Again it could be due to over-activation at both the D1 and D2 receptor, as systemic administration of both a D1 and a D2 agonist, while having no effect on 5-CSRTT accuracy, increased omissions and the

latency to collect the reward (Fernando et al., 2012; Winstanley et al., 2010). This motivational effect is dissociable from the OFC-specific effect of D2 activation, which decreased accuracy (as discussed above) without affecting speed of reward collection, and it probably does not involve DA receptors in the NAC, as D2 agonists infused into this region do not seem to affect either accuracy *or* omissions/reward latencies (Moreno et al., 2013; Pezze et al., 2007). D1 agonists infused into the NAC or mPFC alter accuracy, making effects on omissions/latencies difficult to pinpoint as motivational. So if D1 or D2 receptors are having specific effects on motivation for reward, they are probably located elsewhere.

Another potential mechanism behind a motivationally-induced drop in go responding is the increase in NE observed in the NAC with the co-administration of MPH and FLX, and the corresponding attenuation of MPH-induced peak levels of NE in the PFC observed by Weikop (2007b) (although the area under the curve measurement that they used to compare treatment effects was not significantly different between the combined and the MPH alone treatments). The increase in accumbal NE may be activating α2-adrenergic autoreceptors, which studies with the α2-adrenoceptor agonist guanfacine show reduces levels of extracellular NE in the PFC (Ihalainen & Tanila, 2002;Tanda, Bassareo, & Chiara, 1996a).

α2-adrenoceptor agonists seem to have conflicting effects on action restraint and cancellation. They increase stopping time in the SSRT in rats (Bari et al., 2009; 2011) along with antagonists improving stopping time (Bari & Robbins, 2013). However α2-adrenoceptor agonists reduce rats' PR in the 5-CSRTT (Fernando et al., 2012; Pattij et al., 2012), and human's commission errors in the CPT (Scahill, Chappell, Kim, Schultz, & Al, 2001). This is puzzling, as NET inhibitors improve both action cancellation and restraint. This can be reconciled though, with the observation that measures of omissions and go response latencies were significantly increased by α2-adrenoceptor agonists in these experiments, mostly without affecting accuracy, indicating a potential decrease in motivation, which would of course push PR down and stop latencies up. Results from a markedly different paradigm support this theory, with rhesus monkeys self-administering significantly less cocaine over all doses when acutely administered an α2-adrenoceptor agonist, and also self-administering less food pellets (Kohut, Fivel, & Mello, 2013). The fact that the cocaine dose-response curve showed slightly increased responding to higher doses of cocaine, while cocaine on its own engendered a sharp decrease in responding with higher doses, suggests that the cocaine (and food) had lost some of their reward value, decreasing the animals' motivation to respond.

Aside from the potentiating effects of citalopram on MPH-induced DA and NE levels, MPH was also found to have a potentiating effect on citalopram-induced 5-HT levels, but only in the hippocampus (Weikop et al., 2007b). It is possible that this also may have been a mechanism behind a motivationally-related drop in go accuracy. Globally increasing 5-HT levels via SSRI's has been shown to increase response and reward-collection latencies and increase omissions in the 5-CSRTT, without affecting accuracy (Baarendse & Vandershuren, 2012; Humpston, et al., 2013). Furthermore, global 5-HT depletion, which profoundly reduces 5-HT levels in many regions including the hippocampus, actually has the opposite effect, decreasing or having no effect on omissions, and decreasing response and reward collection latencies in the 5-CSRTT (Carli & Samanin, 2000; Harrison et al., 1997) and go/no-go (Harrison et al., 1999). However because the 5-HT manipulations in these studies induce global change in 5-HT levels, it is only speculation that the specific effects of potentiated 5-HT in the hippocampus could lead to these same behavioural effects.

A final consideration is that the drop in 'go' accuracy in the combined treatment condition mirrored that observed with a high dose (7.5 mg/kg) of MPH alone, which was accompanied by a trend towards a decrease in no-go accuracy, making an attention deficit the most likely explanation. High doses of MPH increase DA levels, but do not affect 5-HT levels in any brain region (Kuczenski & Segal, 2001). Thus the most parsimonious account for both instances of a go-accuracy decrease is a boost in DA transmission causing a lowering of attention.

There are several limitations of the Go/No-Go paradigm used in the current experiment that make it difficult to definitively conclude whether the drop in 'go' responding was due to an effect on motivation or attention. There is no distinct measure of 'omissions' in this task, as these are confounded with successful 'no-go' trials or unsuccessful 'go' trials, and an attentional interpretation. Additionally, in the chambers that were used it is not possible to measure latency to collect the sugar pellet. A possibility would be to measure the latency to respond on the lever, however this would give more of an indication of general arousal than motivation. To gain a clear insight into the motivational effects of MPH combined with FLX, an additional experiment such as progressive ratio self-administration for sugar pellets would need to be run. A decrease in 'breakpoint' (indicating the animal's willingness to work for the reward) would suggest that a deterioration of the reinforcement value of the sugar pellet was the reason for the significantly decreased 'go' responding.

Another limitation of the Go/No-Go paradigm is that the visuo-attentional demands of the task are not particularly high. The rat has only two stimuli to pay attention to, meaning the task is not very sensitive to changes in attentional abilities. As the experiment was chiefly conducted to look at impulsivity effects this was not a major concern, however it would be intriguing to conduct future studies investigating the possible attentional effects of combing MPH and FLX using paradigms that are able to more deeply probe the construct of visual attention, such as the 5-CSRTT. A particularly interesting aspect of attention to investigate, particularly in the context of ADHD, is intra-individual variability of reaction times (Bari & Robbins, 2013). High variability is thought to be indicative of the individual suffering from 'attention lapses' during the task, and has been repeatedly shown to be a cognitive symptom of ADHD patients (Russell et al., 2006). This could be easily measured in either the Go/No-Go task or the 5-CSRTT.

No effects on impulsivity were observed in the Go/No-Go task, under any of the treatments. It was predicted that the low, clinically relevant dose (0.5 mg/kg) would improve 'no-go' accuracy, mirroring the effects observed in humans that have led to MPH being the most commonly prescribed drug for ADHD. That most studies with rodents have not shown an effect of a low MPH dose on action restraint we hypothesised was because these studies overwhelmingly employ the 5-CSRTT, a paradigm that requires a slightly different quality of restraint than the Go/No-Go task, which is most often used to assess action –restraint type impulsivity in humans (see introduction). That we could not demonstrate an improvement in rats' action-restraint with the 0.5 mg/kg MPH dose in the Go/No-Go paradigm either, in agreement with the majority of the studies using the 5-CSRTT, suggests that perhaps methodology is not the reason for the discrepancy between MPH effects in rodents and humans; possibly a more fundamental difference in neural functioning is at play.

However the possibility of low-dose MPH improving rat's action restraint cannot be ruled out by this one experiment. Firstly, mean no-go accuracy did rise from baseline when rats were administered the 0.5 mg/kg MPH dose, although the rise did not reach significance. This rise was especially apparent in the rats with high baseline impulsivity (low no-go scores), with the mean no-go score of the six HI rats increasing from 66 % at baseline to 73.4%, although even the increase for this subgroup did not reach significance. Small numbers of animals and high variability between them may have masked an impulsivity-decreasing effect of low dose MPH, especially in the highly impulsive rats. I.p. injections, especially when administered by novices, can often be misplaced and thus are somewhat

unpredictable in that the drug can sometimes be absorbed IV or SC rather than IP, changing the time the drug takes to absorb and increasing variability between rats. Several sessions of each drug treatment were performed and the scores averaged over these sessions in order to reduce the impact of this problem, but using a larger number of rats would have reduced variability further. A final explanation as to why impulsivity did not decrease after a low dose of MPH is that the majority of these rats appeared to be very distressed at being injected, displaying behaviours such as scratching, hind-paw flinching, high pitched vocalisations, increased defecation and trying to escape (Davar, Hans, Fareed, Sinnott, Strichartz, 1998). It has been shown that acute stress induces a rapid increase of DA in the NAC, (Brake, Zhang, Diorio, Meaney, & Gratton, 2004; Rouge-Pont, Demoche, le Moal, & Piazza 1998) as well as an increase in NE in several areas including the mPFC (Morilak et al., 2005). This could mean that baseline (saline injection) no-go scores were already high, with the potentially impulsivity-lowering effects of a low dose of MPH being mimicked by the injection stress. Thus no-go scores after a 0.5 mg/kg dose would be similar to baseline, explaining the lack of significant improvement seen.

6.2. SSRT experiment

In the SSRT task, which was designed to measure rats' ability to cancel an already initiated action, MPH decreased stopping latency at the 1.0 and 2.5 mg/kg doses, but only for the half of the group comprised of the rats with longer baseline stopping latencies. This was unlikely to be due to a general slowing of responding, as there was no overall effect of MPH on response rate. These results are in agreement with a previous study (Eagle et al., 2007), although these authors also found a stop latency-increasing effect of MPH (showing a detrimental effect on action cancellation) in the rats with fast baseline stopping latencies. None of the doses tested in the current study (up to 7.5 mg/kg) increased stopping latencies for either of the groups of rats. Eagle et al. found the SSRT decreasing effect for the 'slow' group at a dose of 0.3 mg/kg but not 1.0 mg/kg, while at 3.0 mg/kg non-specific effects on arousal were disrupting overall performance for all rats. This is rather different to the dose-effect patterns produced in the current study, for which an SSRT-decreasing effect was not shown at the lowest dose of MPH, (0.5 mg/kg), but was at the higher doses mentioned above. It is not surprising that there are differences in results between the two studies given the differences in methodology. Eagle et al. used a paradigm that was closer to the original 'race-

horse' model of the SSRT, requiring the animal to withhold the second part of a sequential action upon presentation of a stop signal, with stopping latency being estimated by the temporal proximity of the stop signal from the completion of the first part of the sequence during the trials in which they were not able to inhibit their response. Thus, the rats only had 'one chance' to inhibit their response, otherwise they did not receive reinforcement. The rats in the current experiment were able to keep responding after the presentation of the stop signal and still receive a reward, as long as they stopped responding for 3 s *at some time-point* within the LH. They were not punished if they made a response(s), only if they failed to stop responding within the allotted time. Therefore it could be argued that there was less incentive for them to work as hard to stop immediately following presentation of the stop signal, changing what the stop latency represents slightly, and perhaps accounting for the shift of the MPH dose/effect curve rightward in our experiment. Nonetheless, the demonstration that MPH does improve stopping times in our modified SSRT does help to validate it operationally as measuring the same behaviour as the SSRT of Eagle et al. and traditional SSRT tasks used with humans.

FLX also decreased stopping latency at the 1.0 mg/kg dose, but only for the difficult FR2 trials. This is surprising, as previous studies have failed to show an effect of serotonergic manipulations on the SSRT. Rate of responding in the FR2 trials was unaffected by FLX, suggesting that a general slowing of motor activity was not the cause of the decrease. As the decrease was only unmasked in the most difficult trials, it can be classified as a relatively subtle effect, perhaps this is why no decrease in response rate was uncovered.

The 1.0 and 2.5 mg/kg MPH doses, which when administered alone decreased stopping latency, failed to affect stopping latency when administered in combination with 5.0 mg/kg FLX. This is contrary to our hypothesis that administering FLX alongside MPH would not change the effects of MPH alone, or possibly potentiate them due to an increase in NE levels. Rather, these results indicate an attenuating effect of the FLX. However this could be symptomatic of the apparent practice effect present in the experiment, with rats' baseline stopping latency significantly decreasing in between the MPH doses and the combined doses. As the baseline measure for the combined treatments was already a fast stopping latency, it may have been resistant to improvement, showing a ceiling effect.

The 0.5 mg/kg dose of MPH, which did not affect stopping latency on its own, also did not affect this measure when combined with 5.0 mg/kg FLX, however although it failed

to reach significance, the mean latency produced by the combination treatment was faster than baseline. Considering that the baselines for the combined treatment were much quicker due to the practice effect mentioned above, leaving little room for improvement, it is conceivable that the decrease may have reached significance, had the combined treatment sessions been administered closer to the 0.5 mg/kg MPH sessions, allowing them to share the saline 1 baseline.

The presence of the practice effect makes comparing treatments very difficult, and the results unclear and difficult to interpret. Counterbalancing would have reduced the confounding practice effect, but with this small number of rats it may have eliminated all treatment effects completely. It would be interesting to repeat this experiment with a larger *n* and counterbalanced treatments to investigate whether the addition of an SSRI to MPH can in fact enhance its impulsivity-reducing effects, for the sub-type of impulsivity termed 'action cancellation'.

6.3. Locomotor activity experiment

Neither the 0.5 nor 2.5 mg/kg doses of MPH given alone affected baseline locomotor activity, nor did the 5.0 mg/kg dose of FLX administered alone. However when the 2.5 mg/kg MPH dose was co-administered with the FLX, activity significantly increased compared to baseline, and the singular administration of the two drugs. Activity in this condition remained significantly raised over minutes 5-35, while slowly decreasing, and by 40 minutes post- injection, had decreased to around the same level as the other treatments. The combination of the 0.5 mg/kg MPH dose with FLX did not result in an increase in activity. These results fit in well with the reported enhancement in accumbal DA levels when 2.5 mg/kg MPH was administered alongside citalopram (Weikop, 2007b).

It is extremely unlikely that that the increase seen in the 2.5 mg/kg MPH + 5.0 mg/kg FLX condition was due to an additive effect of the two drugs, as while the mean for the 2.5 mg/kg MPH treatment was slightly, but not significantly higher than baseline, the mean for the FLX dose was slightly below baseline. It seems much more plausible that this was due to a synergistic effect of the two drugs. This result extends previous findings by Borycz et al. (2008) who found the same effect but used a much higher MPH dose (10 mg/kg). However it contradicts Weikop et al. (2007b), who did not find a locomotor-potentiating effect of the two

drugs in combination. Although the same doses were used in the Weikop study, they were administered via subcutaneous (s.c.) injection, which has a slower absorption rate compared to i.p. 2.5 mg/kg MPH i.p. induces a peak rise in accumbal DA within the first 40 minutes post-injection (Gerasimov et al., 2000; Kuczenski & Segal, 2001), whereas s.c. administration of this dose produced a peak in accumbal DA after 80 minutes (Weikop et al., 2007b). This is unlikely to be a factor however, as the drugs were administered 80 and 60 minutes before activity recording, with movement recorded over a period that corresponded with potentiated dopamine release in the NAC. Probably the most likely reason for the different outcomes of the current study and that of Weikop et al. (2007b) is that our rats received a shorter habituation period. By the time DA levels were at their peak (60-80 minutes after the second injection) in the Weikop study, showing that the drug treatments had taken effect in the CNS, rats had already had 60 minutes to habituate to their chambers. This is when activity recording started. It is very likely that the rats in our study were not fully habituated at the time recording started, with the habituation period prior to the injections being 30 minutes, and recording starting five minutes post injection, giving a total of 35 minutes habituation. If our rats were still displaying novelty-induced heightened activity at the beginning of the activity recording (and the higher baseline activity count in our experiment compared to Weikop's suggests this) we may have captured a different treatment effect, one that is only present in less habituated rats. Borycz et al. (2008), despite giving rats a 3.5 hour habituation period, also began recording directly after giving the second injection, which could explain the similarity in results to our study.

The level to which rats are habituated does alter locomotor responses to drug treatments. AMPH or cocaine given in a novel environment increases rats locomotor response compared to administration in a familiar environment, with this difference becoming more pronounced over a course of injections, suggesting that novelty increases sensitisation to stimulant drugs (Badiani, Anagnostaras, & Robinson 1995a; Badiani, Browman, & Robinson, 1995b; Badiani et al., 2000). Drugs can even have the opposite effects depending on habituation, for example 8-OH-DPAT supresses activity in animals who are not habituated to the chamber, but increases activity in animals who have been habituated for two hours (Evenden & Angeby-Moller, 1990). As this compound significantly increased activity in the Weikop study, we can assume that their rats were well habituated. On the contrary, in our experiment, the administration of FLX, which acutely activates 5-HT1A receptors (see below), decreased activity (although not significantly), which suggests that our

rats were not well habituated. Why would unhabituated, i.e. more active animals react differently to the combination of 2.5 mg/kg MPH + 5.0 mg/kg FLX?

It has been shown that exposure to a novel environment increases DA levels in the NAC (Legault & Wise, 2001), so it is conceivable that the already heightened mesolimbic DA activity induced by the novel chamber may speed and strengthen the potentiating effect of FLX on MPH-induced DA outflow in the NAC. Although the potentiation of AMPH-induced locomotor activity by a novel environment has not been found to correspond with enhanced DA activity (Badiani et al., 1998, 2000), the combination of the SSRI with a stimulant AND a novel environment in the current experiment may have produced a difference outcome, although the mechanism behind this possible interaction remains unknown.

The other factor that may have enhanced the locomotor response to the combination treatment is the level of stress displayed by the rats when being injected. This is supported by the temporal proximity of the rise in activity induced by the combination treatment to the time of injection. The rats used in this study seemed to be particularly distressed by injections compared to other rats observed in the laboratory (although of course the amount of stress displayed by the rats in the Weikop study is unknown). As discussed above, acute stress induces a rapid increase of DA in the NAC, which peaks after about 15 minutes (Brake et al., 2004; Rouge-Pont et al., 1998). Although all the treatment conditions received the injection(s) at the same time-point, only the 2.5 mg/kg MPH + 5.0 mg/kg FLX condition showed the increase in locomotor activity, suggesting that the potentiation of DA release by the FLX added to the DA increase induced by the stressful injection enough to affect locomotor activation. Going further than acute stressors, chronic stress can induce long term sensitisation to the locomotor effects induced by drugs such as AMPH, cocaine and morphine (Bisagno et al., 2004; Del Rosario, Pacchioni, & Cancela, 2002; Haile, GrandPre, & Kosten, 2001) and also potentiate stimulant-induced DA efflux (Sorg & Kalivas, 1991). It could be argued that the rats in the current study were exposed to chronic stress as they were injected twice a week over the course of several months, showing signs of elevated stress each time they were injected, leading to a sensitised response to the combination treatment.

6.4. Possible mechanisms for the synergistic effects of FLX and MPH on DA release and thus on attention and locomotion.

Although neurotransmitter levels were not investigated in the current study, based on the previous research it can be assumed that the observed synergistic effects of MPH and FLX on attention and locomotor activity are likely to be the result of an increase in DA levels in the NAC and PFC.

As no receptor-specific manipulations were conducted in the present study, we can only speculate on the mechanism behind this potentiation of dopamine activity based on other research. Acutely administered SSRI's have quite different effects on the 5-HT system compared the effects seen after a period of chronic administration. Acutely, they produce an overall decrease in 5-HT transmission due to the strong activation of 5-HT1A autoreceptors in the DRN (Artigas, Romero, de Montigny, Blier, 1996; Blier & de Montigny, 1994) and 5-HT1B autoreceptors (Artigas, 2013). It takes several weeks of daily administration for the neuro-adaptations to occur that allow SSRI's to induce an increase in extracellular 5-HT (Tanda, Frau, & Chiara, 1996b), however the administration of 5-HT1A and 1B antagonists alongside SSRI's allow for a much quicker increase in 5-HT as the initial negative feedback system is blocked (Gobert, Rivet, Cistarelli, & Millan, 1997; Hervas, Queiroz, Adell, & Artigas, 2000; Portella et al., 2011). In the introduction it was discussed how each of these 5-HT receptors influences DA activity, with activation of the 5-HT1A autoreceptor increasing activity and DA levels in structures such as the PFC, VTA, NAC, SN and striatum, and 1B receptors in the hippocampus disinhibiting DA release in the NAC through glutamatergic and GABAergic connections, making these receptors likely candidates for the mechanism behind the synergistic effect of FLX and MPH on dopamine activity and associated behaviours. This is in line with demonstrations that antagonism of these receptors blocks the effect (Borycz et al., 2008; Weikop et al, 2007a).

The acute effect of SSRI's on the 2A and 2C receptors, which also influence DA activity, are less well-known. However, 2C antagonists, like 1A and 1B, accelerate the eventual 5-HT enhancing effects of SSRIs (Cremers et al., 2004, 2007; Opal et al., 2014), suggesting that they may be similarly activated by acute SSRI administration, and a 2A/C antagonist blocks the EPS potentiating effects of acute FLX on haloperidol, suggesting that FLX acutely activates 2C receptors, which would inhibit DA release in the striatum (Tatara et al., 2012). SSRI-induced activation of 2C receptors would not be contributing to the

potentiation of MPH- induced DA release in the NAC and PFC as these receptors inhibit both basal and activated accumbal DA activity (see introduction, section 7.3).

A limitation of the current study is that it looked only at effects of acutely administered drugs. Twice-weekly administration of MPH over several months could possibly be considered chronic, however, doses and treatment conditions would needed to have been administered twice, at the beginning and end of this period, to examine differences between chronic vs. acute administration. However the administration of FLX in this study cannot be considered chronic, with gaps of a week or more in between injections. When administered chronically, FLX has substantially different actions to its acute administration (described above). These are made possible by via neuro-adaptations involving the desensitisation of the 5-HT 1A and 1B autoreceptors (Blier & de Montigny, 1994; Hervas et al., 2001, Neumaier, Root, & Hamblin, 1996). 2A and 2C receptors have also been shown to be downregulated after chronic SSRI administration (Artigas, 2013; Gray & Roth, 2001). The extracellular 5-HT-elevating actions of chronic SSRI's are thought to be partly mediated by tonic activation of postsynaptic 5-HT1A receptors in the hippocampus (Blier & Ward, 2003; Haddjeri et al., 1998).

If the potentiating effect of FLX on MPH-induced DA release is due to its acute actions at the 1A and 1B receptors, then chronic administration of FLX together with MPH is not likely to produce this effect, or a different synergistic effect may emerge.

So when speculating on the application of the results of this study to humans taking both MPH and FLX, it must be noted that if the behavioural effects observed in the study (lowered attention and hyperactivity) transfer to humans, they would be likely only to occur with the first few doses of SSRI's, for which acute effects would still be present.

6.5. Conclusions

The results of this study provide important evidence that the combination of MPH and FLX can synergistically influence aspects of behaviour in a way that differs to their effects when administered alone. This builds on previous research that has shown that the neurochemical effect of co-administration of these two drugs differs from their singular administration.

It was found that when 2.5 mg/kg of MPH was administered alongside 5.0 mg/kg of FLX, 'go' response accuracy diminished, mirroring the effect of 7.5 mg/kg MPH alone. This could indicate a drop in attention or motivation. It was also found that this same combination of drug doses induced a rapid increase in open-field locomotor activity, an effect not seen with either drug administered singly. This locomotor-increasing effect has been shown in a previous study, but with a much higher dose of MPH (10.0 mg/kg).

Although a 2.5 mg/kg dose of MPH is higher than a human therapeutic dose, there still may be important implications of these results for people who are prescribed both a stimulant and an antidepressant. That the drugs have an observable synergistic effect at these doses means it is feasible that the effect may still be present, albeit expressed more subtly, at therapeutic doses. Sensitive, fine-grained cognitive testing in human populations could reveal these effects. ADHD patients, who are likely to already be suffering an attention deficit or hyperactive, may be particularly vulnerable to these effects.

Furthermore, we have trialled a new type of SSRT for rodents that is much simpler to run and analyse. Stop latencies were modulated by trial difficulty, and MPH shortened stop latencies, supporting its operational validity.

Although no clear effects of co-administering the two drugs were found on either subtype of impulsivity (action restraint or cancellation) more studies are needed to rule out the possibility that SSRI's may alter the impulsivity-reducing effects of MPH, preferably using the same paradigms on human participants, for whom the impulsivity reducing effects of MPH are much more robust than in rodents.

This study has investigated three out of a huge number of behaviours and cognitive processes that are moderated by DA transmission. That we have demonstrated a synergistic effect of MPH and FLX on some of these processes should encourage further studies examining the effects of these two drugs in combination, for example on animal models of drug abuse such as self-administration. As the number of new patients being prescribed both stimulants and SSRI's rises, more information needs to be uncovered regarding potentially harmful synergistic behavioural and cognitive effects of these drugs.

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