Pharmacology & Therapeutics 158 (2016) 41–51

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate Editor: F. Tarazi

Low attentive and high impulsive rats: A translational animal model of ADHD and disorders of attention and impulse control

Andrew Hayward [a,*], Anneka Tomlinson [b], Joanna C. Neill [a,*]

[a] Manchester Pharmacy School, University of Manchester, Manchester M13 9PT, UK
[b] Green Templeton College, University of Oxford, Oxford OX2 6HG, UK

ARTICLE INFO

Available online 23 November 2015

Keywords:
Animal model
ADHD
5C-SRTT
5C-CPT
High Performance
Low Performance

ABSTRACT

Many human conditions such as attention deficit hyperactivity disorder (ADHD), schizophrenia and drug abuse are characterised by deficits in attention and impulse control. Carefully validated animal models are required to enhance our understanding of the pathophysiology of these disorders, enabling development of improved pharmacotherapy. Recent models have attempted to recreate the psychopathology of these conditions using chemical lesions or genetic manipulations. In a diverse population, where the aetiology is not fully understood and is multifactorial, these methods are restricted in their ability to identify novel targets for drug discovery. Two tasks of visual attention and impulsive action typically used in rodents and based on the human continuous performance task (CPT) include, the well-established 5 choice serial reaction time task (5C-SRTT) and the more recently validated, 5 choice continuous performance task (5C-CPT) which provides enhanced translational value. We suggest that separating animals by behavioural performance into high and low attentive and impulsivity cohorts using established parameters in these tasks offers a model with enhanced translational value. In this review, methods to separate animals are compared and the results discussed to highlight advantages over more constrained models, in addition to potential future directions for enhanced validation. Advantages include reliability, flexibility and enhanced translation to clinical conditions, all important considerations in modelling ADHD, schizophrenia and drug abuse, conditions with multifactorial aetiology. Based on the existing evidence, we suggest that future studies should incorporate an element of behavioural separation when studying the constructs of visual attention and impulsive action of relevance to human disorders.

Contents

Abbreviations: 5C-CPT, 5 choice continuous performance task; 5C-SRTT, 5 choice serial reaction time task; Acc, accuracy; ADHD, attention deficit hyperactivity disorder; ADHD-C, ADHD combined subtype; ADHD-I, ADHD inattentive subtype; COMT, catechol-o-methyl transferase; CPT, continuous performance task; DAT, dopamine transporter; DRD4, dopamine D_4 receptor; DA, dopamine; E, efficient performers; HA, high attentive; HI, high impulsive; HP, high performance; LH, lister hooded; IA, inattentive; IA-I, IA impulsive; ITI, inter-trial interval; LA, low attentive; LI, low impulsive; LP, low performance; MPH, methylphenidate; M, moderate performers; NA, noradrenaline; NAT, noradrenaline transporter; NAc, nucleus accumbens; NAcC, nucleus accumbens core; NAcS, nucleus accumbens shell; OFC, orbital frontal cortex; PrL, prelimbic cortex; PR, premature responses; pFA, probability of false alarms; SI, sensitivity index; SD, stimulus duration.

* Corresponding authors.
E-mail addresses: andrew.hayward@manchester.ac.uk (A. Hayward), joanna.neill@manchester.ac.uk (J.C. Neill).

http://dx.doi.org/10.1016/j.pharmthera.2015.11.010
0163-7258/

1. Introduction

The focus of this review is attention deficit hyperactivity disorder (ADHD) as an illness clearly characterised by abnormal hyperactivity as well as deficits in attention and impulsivity, although many of the constructs we discuss are relevant for other disorders such as drug abuse and schizophrenia.

Psychiatric disorders remain poorly managed and present a large economic burden. Brain disorders cost €141 billion per annum in the UK, with a total 2010 cost (in million € purchasing power parity) of € 134 (Fineberg et al., 2013). ADHD is often confounded by other conditions such as generalised anxiety disorder, oppositional defiant disorder and learning difficulties (Larsson et al., 2011). The estimated direct and indirect costs per child or adolescent patient are estimated to be €9,860 to €14,483 per year (Le et al., 2014). Of this, between 8–25% are estimated to be direct healthcare costs, showing that a large proportion of the economic cost is due to indirect costs such as education and social services. When the condition persists into adulthood, symptoms begin to include poor occupational performance (Kuriyan et al., 2013) and higher risk taking behaviour (Flory et al., 2006; Groen et al., 2013) resulting in even poorer financial and societal outcomes (Doshi et al., 2012). Factors such as these contribute to a reduced quality of life for ADHD patients, which often persist through middle age into old age if left untreated (Brod et al., 2012).

ADHD affects 5–8% of school aged children and can persist to adulthood in 50–80% of cases (Barkley et al., 1990; Faraone et al., 2000; Fayyad et al., 2007; Bari et al., 2008). It is recognised as a heterogeneous disorder characterised by three main symptoms (inattention, hyperactivity and impulsivity), which forms the basis of the current psychiatric classification system. However a recent review describes the many other different methods of characterising ADHD, including; types and traits, age, gender, treatment, duration and variations in neuroanatomical structure and function (Heidbreder, 2015). ADHD is most commonly sub-classified as; 1. Predominantly inattentive (ADHD-I), 2. Predominantly hyperactive/impulsive (ADHD-HI) 3. Combined type presentation (ADHD-C). In a large meta-analysis investigating the validity of the subtypes and symptom dimensions, the authors concluded that separation into subtypes is a useful, convenient way to describe the functional and behavioural aspects of ADHD, however care must be taken when defining the subtypes as discrete distinct forms of the disorder (Willcutt et al., 2012).

There is a large evidence-base confirming the heterogeneity between subtypes in ADHD. This evidence suggests the existence of neurocognitive, gender, neural correlate and behavioural differences (different phenotypes) between subtypes. The gender differences reported show that women are more likely than men to be diagnosed with the inattentive subtype of ADHD (Biederman et al., 2002) and men are more likely to be diagnosed with the combined subtype (Ramtekkar et al., 2010). Interestingly, the male to female ratio differs between children and adults. In children, ratio estimates differ depending on the sample; in community surveys male to female ratio is higher (as high as 10:1) than in a clinical sample (as low as 3:1). However in adults, this ratio is reduced (1:1 to 2:1) (Williamson & Johnston, 2015). The importance of recognising gender differences in research is highlighted in a recent comprehensive review, which recommends adequate representation of both genders in clinical samples (Williamson & Johnston, 2015). It is vital that both male and female subjects are included in future studies, including in animal studies to represent the true clinical picture of ADHD and to more carefully assess the contribution of gender to the pathophysiology of the disease.

The ADHD-200 Consortium investigating heterogeneity in ADHD has shown a number of specific neural correlates within the subtypes of ADHD (ADHD-200-Consortium, 2012). The ADHD-I subtype was found to have variations in specific brain regions including mainly dorsolateral prefrontal and cerebellar regions, known to be associated with control systems. ADHD-C subtype patients were noted to have atypical connectivity in midline default network components and the insular cortex. These results support the hypothesis that differential connectivity disturbances underpin each ADHD subtype (Fair et al., 2013). It has also been shown that an abnormality in the motor circuit represents the major difference between ADHD-C and ADHD-I subtypes (Lei et al., 2014).

Existing pharmacotherapy is not designed for the diversity of symptom presentations, and therefore lacks efficacy and also has a large side effect burden. Critical to the development of improved therapy is improved understanding of the aetiology and neurobiology of these disorders, achieved through carefully validated animal models, which are currently lacking.

Stimulant medication such as methylphenidate and amphetamine are currently first and third line treatment in the national institute of clinical excellence (NICE) guidelines respectively (Palanivel et al., 2009). They control inattentive symptoms in approximately 75% of patients, but do not adequately control impulsive symptoms and, at high doses and in certain patient groups, may even exacerbate them (Faraone & Antshel, 2008; Winstanley, 2011). They are also contraindicated for patients with a history of drug abuse due to their abuse liability. The second line treatment is the non-stimulant, atomoxetine which alleviates impulsive measures in the continuous performance, stop signal, and delay discounting tasks in humans (Chamberlain et al., 2006; Barry et al., 2009), however, for attentive measures, response rates and effect sizes are lower than for stimulants (Heal et al., 2009; Cunill et al., 2013). A recent meta-analysis of atomoxetine treatment concluded that the risk–benefit ratio for atomoxetine is even lower in an adult ADHD population as it has only minimal effect on clinically meaningful endpoints (e.g., job performance or sociability) (Cunill et al., 2013). Other non-stimulant treatments, guanfacine and clonidine are only recommended in North America for ADHD (Bolea-Alamanac et al., 2014). Their omission from NICE and other European guidelines is likely due to the need for clearer evidence of their efficacy across patient groups, particularly in adulthood. With adult ADHD being increasingly recognised to have a significant socioeconomic burden, it is important that we find new pharmacological treatments with improved efficacy, safety and tolerability in the adult ADHD population, or enhance the use of available medication by delivering the most appropriate treatment to each subtype of patient (Kooij et al., 2010). Less commonly used treatments include guanficine, modafinil, desipramine, and bupropion (Palanivel et al., 2009; Minzenberg, 2012). These drugs are not recommended by NICE as their mechanism of action is not yet fully understood. Taking all this into account, it is important to conduct further research using animal models in order to define patient populations with symptom profiles who may benefit most from the different types of medication.

1.1. Value of animal models and the need for improved translation

Animal models offer the ability to control and manipulate experimental parameters more precisely and rapidly than in human subjects (Markou et al., 2009; Moore, 2010). This level of manipulation supports hypothesis driven research, with the overall aim of improving understanding of neuropathology, and developing better treatments for clinical conditions. The high attrition rate of drug development programmes and the withdrawal of the pharmaceutical industry from psychiatric drug discovery has been attributed to poor translation from the animal models into the clinic (Nutt & Goodwin, 2011; Insel & Sahakian, 2012; Neill & Hendrie, 2012). However, a more likely explanation is that not enough time and resources have been provided to improve validity of the animal models and appropriateness of the tests employed, a situation currently being addressed (eg see Neill et al., 2014; Stuart et al., 2013; and Tomlinson et al., 2014 for ADHD). Progress in this area is essential to enable much needed advancement in psychiatric drug development. This section of our

review aims to summarise recent efforts to enhance translation from rodents to the clinic in ADHD, using the separation of rodents into high and low performing groups based on attentive and impulsive measures. The tests used for this include the standard 5-choice serial reaction time task (5C-SRTT) and a task with enhanced translation, the 5-choice continuous performance task (5C-CPT).

There are three factors suggested by Willner (1986) to consider when reviewing an animal model: face, construct and predictive validity. Firstly, face validity requires the model to produce the same phenotype as the human condition. In the case of ADHD, deficits in measures designed to translate to human attention and impulsivity are key to producing the model. Furthermore, models designed to align with clinical subtypes further increase this translational power. Secondly, construct validity is required to mimic aetiology and neurobiology of the human condition in the animal. ADHD as an example is considered highly heritable, with estimates of 80% or greater heritability (Biederman et al., 1990; Kieling et al., 2008; Langner et al., 2013). Multiple genetic factors have been linked to this heritability such as a dopamine D4 receptor (DRD4) seven transmembrane repeat (Faraone et al., 2001; Li et al., 2006; Gornick et al., 2007; Woolley et al., 2008) and the dopamine transporter type 1 (Lim et al., 2006). This suggests that several genetic factors combine to produce the observed phenotype, or that other factors may play a role including environmental factors and epigenetics (Maher, 2008). Due to the multifactorial and diverse nature of the aetiology of ADHD and many other neuropsychiatric conditions, etiological genetic modelling would be a restrictive method preventing exploration of alternative mechanisms (Nestler & Hyman, 2010; Bari & Robbins, 2011). One alternative is lesion modelling, but this suffers the same issue of restriction of mechanism, and lesion or manipulation of brain regions with neurotoxins only mimics a small group of ADHD patients who encounter toxins such as heavy metals. Ultimately these methods investigate the effect of the manipulation rather than attempting to replicate the core symptoms of ADHD (Jupp et al., 2013).

One theory of ADHD, and indeed other disorders, is that it represents the extreme of a continuum present within the general population (Bari & Robbins, 2011). As rats tested in the 5C-SRTT and 5C-CPT show a continuum of performance, a method of modelling with stronger validity and the potential for translation is behavioural separation based upon differences in performance, as it avoids assumption of mechanisms (Puumala et al., 1996; Dalley et al., 2007; Tomlinson et al., 2014). A variety of separation methods have been used to produce differing models based on the condition under investigation or hypothesis studied. Taking into account the important differences between the subtypes identified by behavioural separation, optimum animal models need to characterise specific symptoms (i.e., impulsivity) or model the individual subtypes of ADHD. We review the main methods used to date and assess their translational value in the following sections.

1.2. Measuring attention and impulsive action in rodents

In humans, the CPT is the most widely used task to measure sustained attention (errors of omission and accuracy of response), impulsive action (errors of commission) and vigilance (d prime-d′), the ability to remain aware of changing situations in order to respond correctly (Riccio et al., 2002; Epstein et al., 2003). All of these are readily measured in animals. The most widely used test is the rodent 5C-SRTT (Carli et al., 1983; Robbins, 2002; Bari et al., 2008). This test has recently been further validated by reverse-translation to humans, as the four choice serial reaction time task (Voon et al., 2014). For full details of the methods for 5C-SRTT see Bari et al. (2008), in brief a rodent, usually a rat, is placed into a standard five-hole operant chamber (Fig. 1). During the task a light stimulus is lit briefly in the back of a randomly selected aperture. The animal's ability to detect and respond, usually via a nose poke response, to the correct aperture is recorded as a measure of accuracy, which is considered an analogue of selective attention (Robbins, 2002). A second measure provided by this task is premature responding during the waiting period, before the next stimulus presentation. This is considered a measure of impulsive action and will be termed waiting impulsivity for the purposes of this review. This task has been widely adopted as a preclinical task of attention and waiting impulsivity (Robbins, 2002; Winstanley, 2011).

A variation of this task is the 5 choice continuous performance task (5C-CPT) first described in mice (Young et al., 2009), and recently validated in our laboratory for rats (Barnes et al., 2012a, 2012b). It has also been reverse translated to humans, demonstrating translational validity (McKenna et al., 2013). In the CPT, the same response to a single light stimulus is required during most trials, but infrequent non-target trials add an additional challenge. In non-target trials, the pre-potent signal of all five lights requires no response by the animal in order to gain a food reward (i.e., the animal is rewarded for withholding its impulse to respond). Responding during this trial, rather than withholding responding, is termed a false alarm, and interpreted as response disinhibition (Fig. 1). Response disinhibition closely corresponds with human errors of commission in the CPT (Riccio et al., 2002). Response disinhibition is a form of impulsive action, but is genetically and pharmacologically distinct from waiting impulsivity as measured by premature responses in the 5C-SRTT and 5C-CPT (Young et al., 2011). When DRD4 expression is reduced, response disinhibition increases without affecting the premature responses. Pharmacologically, SD242084 (a 5-HT_{2C} receptor antagonist) increases

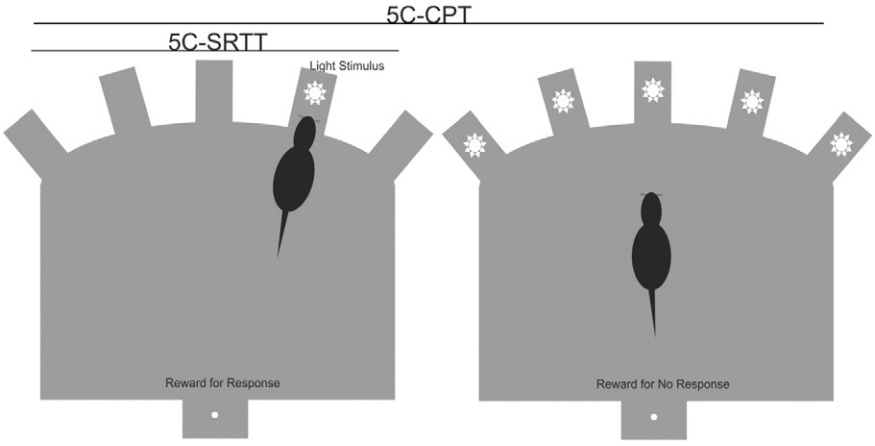

Fig. 1. The 5 choice serial reaction time task, left panel (5C-SRTT) only uses trials where a reward is earned by responding in the hole in which a light stimulus appears, either during the stimulus duration, or immediately afterwards, in the remaining limited hold period. The 5 choice continuous performance task (5C-CPT) uses these target trials and adds infrequent non target trials where all five lights are lit and no response is required to earn a food reward, no go trials are shown in the right panel.
Adapted with permission from Tomlinson et al. (2015).

premature responses without affecting false alarms. The addition of non-target trials also enables the assessment of vigilance (measured as sensitivity index-SI) by robust statistical analyses using signal detection theory in the same way as in the human CPT (discussed in detail in Young et al. (2009)). The 5C-CPT in rodents offers enhanced translation to humans because of its close resemblance to the human CPT. Due to this enhanced translational value and lengthy training procedure, it has been suggested that the 5C-CPT is best used at later stages of the drug development process and that the 5C-SRTT is more suited to earlier stages, due to its higher throughput (Lustig et al., 2013).

2. Methods of separation

2.1. Models of high impulsive action

The most commonly used method was first described by Dalley et al. (2007) and has been used in several studies since, demonstrating its reproducibility (Table 1). This method involves separating rats based on premature responses in the 5C-SRTT, as an analogue of impulsive action. Dalley and colleagues use adult male Lister Hooded rats in this model. In order to separate the animals, they were trained to standard parameters of the task (as above) using a 5 seconds (s) fixed inter-trial interval (ITI), they were then cycled through 3 weeks of daily testing with two days at a 5 s fixed ITI, one day at a 7 s fixed ITI and then two days at a 5 s fixed ITI again (Fig. 2). The use of two ITIs demonstrates the rats' performance at baseline (5 s) and when challenged (7 s) (Dalley et al., 2007). High Impulsive (HI) animals were originally shown to have twice as many premature responses as Low Impulsive (LI) animals at 7 s ITI (Dalley et al., 2007). They have also been characterised as having 50% premature responses across the three weeks at 7 s ITI with LI animals having less than 30% premature responses (Caprioli et al., 2013, 2014) or the HI as being in the top 15th percentile for impulsivity (Isherwood et al., 2015). This HI model was originally used to model aspects of drug taking behaviour, such as abstinence and relapse in addition to investigation of the neuroanatomical basis of impulsive behaviour, more specifically, the role of prefrontal cortex and ventral striatum circuitry in impulsive behaviour (Jupp et al., 2013). This method results in approximately 7% of the animals being classified as HI, which is then a stable trait when retested over time (Dalley et al., 2007).

Another method of separation for impulsivity is described by Diergaarde et al. (2008) who separated a group of 32 male Wistar rats based on premature responding by upper and lower quartiles using five stable training sessions (Table 1). This is a shorter baseline period than that used by Dalley et al. (2007). Training sessions used a standard 5 s fixed ITI but a 1 s stimulus duration (SD), which is longer than the standard SD (Bari et al., 2008). The primary effect of altered SD is on accuracy and, as the limited hold is 1 s in both cases, there would be an expected minimal effect on premature responses. This method produced 8 HI and 8 LI animals, removing 16 moderately performing animals from the comparison. This model has been used to study nicotine dependence and non-drug related addiction by sucrose self-administration (discussed later). The advantage of this method is that it offers a simple means of separation with a substantial difference in performance between the subgroups. While use of cohort characteristics such as upper and lower quartile makes comparison within a cohort robust, it also means that whole cohort performance is an important factor when comparing between studies.

Winstanley et al. (2010) used a similar method, 41 male Long-Evans rats were trained to a stable five-day baseline in the 5C-SRTT (Table 1). They were then separated according to the median premature responses. Using the median reduces the effect of extreme values and allows re-establishment of a baseline between experiments. However, it also produces an unequal HI:LI ratio. The authors state that this method was not designed to rigorously separate animals into extreme groups but to offer a simple separation method and also has the ethical benefit of utilising all members of a cohort (Winstanley et al., 2010). This model has been used to study obsessive compulsive disorder and bipolar disorder.

Tomlinson et al. (2014) recently proposed a model to account for impulsive symptoms by using the parameters of premature responses (waiting impulsivity) and probability of false alarms (pFA; response inhibition) offering a unique modelling method using the highly translatable 5C-CPT in female Lister Hooded rats (Table 1). For this model, the parameters were premature responses above or below 10 and probability of false alarms above or below 0.5 for high and low impulsive groups respectively. As response inhibition is a critical factor of ADHD impulsive and combined subtype presentation in people (Epstein et al., 2001) this model is designed for enhanced translation to the clinical CPT (Young et al., 2009; Lustig et al., 2013; Tomlinson et al., 2014, 2015).

Table 1
Summary of published methods for modelling impulsivity, attention and combined symptom subtypes using performance based separation techniques, as discussed above. Abbreviations: 5 choice-serial reaction time task (5C-SRTT), 5 choice continuous performance task (5C-CPT), high/ low impulsive (HI/LI), high/ low performance (HP/LP), attention deficit hyperactivity disorder (ADHD), ADHD inattentive subtype (ADHD-I), ADHD combined subtype (ADHD-C), inattentive (IA), IA impulsive (IA-I), moderate performers (M), efficient performers (E), Lister Hooded (LH), premature response (PR), probability of false alarms (p[FA]), accuracy (Acc), sensitivity index (SI).

	Developed by	Test	Model	Rat sex & strain	Parameter	Rule	Additional notes
Impulsivity	Dalley et al. (2007)	5C-SRTT	HI	Male LH	PR	>2x at 7 s ITI	Tested over 3 weeks
	Diergaarde et al. (2008)		HI/LI	Male Wistar	PR	Upper/lower quartiles	
	Winstanley et al. (2010)		HI/LI	Male Long-Evans	PR	Above/below median	
	Tomlinson et al. (2014)	5C-CPT	HI/LI	Female LH	PR	>/<10	Mean of 5 tests
					p[FA]	>/<0.5	
Attention	Granon et al. (2000)	5C-SRTT	HP/LP	Male LH	Acc	>/<75% accuracy	When stable over 5 sessions
	Paterson et al. (2011)		LP	Male Long–Evans	Acc	<75% accuracy and >50 trials	Similar to Granon et al. (2000), but only used LP group
	Grottick and Higgins (2000)		LP	Male LH	Acc	<75%	Stable for 2 weeks.
					Omissions	>20%	
	Tomlinson et al. (2014)	5C-CPT	ADHD-I/control	Female LH	Acc	>/ < 90%	Mean of 5 tests
					SI	>/ < 0.3	
Attention & Impulsivity	Puumala et al. (1996)	5C-SRTT	ADHD/control	Male Han:Wistar	Acc	<60%/>75%	
					PR	>40%/<30%	
	Blondeau and Dellu-Hagedorn (2007)		IA-I/IA/M/E	Male Sprague–Dawley	Acc	Cluster analysis	Control and three subtypes of ADHD
					PR		
	Tomlinson et al. (2015)	5C-CPT	ADHD-C/control	Female LH	Acc	</>90%	Attention, vigilance and impulsive action
					SI	</>0.3	
					p[FA]	>/<0.5	

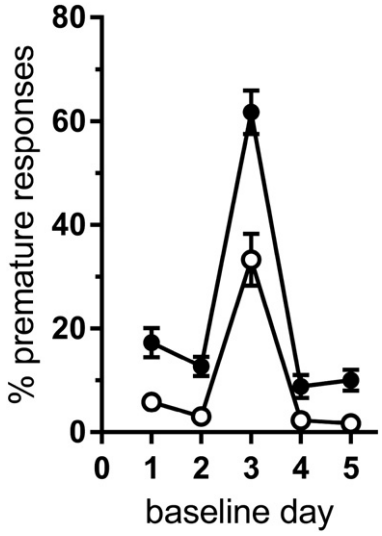

Fig. 2. Baseline separation of HI-filled circles/LI-empty circle animals using the Dalley et al. (2007) method. This establishes a baseline of 5 s ITI and then challenges the animals with 7 s long ITI. Under this challenge, HI animals can be separated. The 5 day schedule is repeated for three consecutive weeks for reliability.
Reproduced with permission from Dalley et al. (2007).

2.2. Models of low attention

To separate animals for attentive performance, Granon et al. (2000) used the measure of accuracy and selected animals performing above or below 75% accuracy to produce high and low baseline performance groups respectively (Table 1). Male Lister Hooded rats were trained to 5 s SD as in the standard 5C-SRTT protocol (Bari et al., 2008). Once rats had been trained, stability of performance was measured over a five day baseline. Animals in which performance deviated <5% from the mean were progressed to a reduced SD (not lower than 0.25 s). Before testing, animals performed stably between 0.5–0.25 s SD, this varied between animals so is a confounding factor for analysis, but high attentive (HA) and low attentive (LA) groups were well balanced in SD. Ensuring stability is important for accurate interpretation of the results, but testing the animals based on different SDs alters the nature of the separation as the ability to respond at shorter SDs is seen as an attentive trait which directly affects accuracy (Robbins, 2002; Young et al., 2013).

Paterson et al. (2011) used male Long Evans rats to produce a suboptimally performing group (Table 1). This, as with the previous model, was based on less than 75% accuracy across a 5 day baseline, but this study only looked at the low performing group. To control for lack of trials completed they also stipulated that at least 50 trials had to be completed in each of five consecutive sessions. They state that 25% of the whole group of rats met these criteria making it similar to a lower quartile separation.

Grottick and Higgins (2000) produced a model to test the hypothesis that nicotine has a pro-attentive effect that cannot be measured in normal animals using the 5C-SRTT (Table 1). They chose male Lister Hooded rats with < 80% accuracy and > 20% omissions. They are the only group to separate using omissions as a measure of ability to sustain attention. Although it is debated whether omissions represent sustained attention or motivation in this task (Robbins, 2002), this demonstrates the flexibility to separate by a particular parameter of interest.

The 5C-CPT has also been used recently to model the attentive deficits in ADHD, which are known to persist into adulthood more consistently than impulsive symptoms (Ingram et al., 1999; Wilens et al., 2004; Kooij et al., 2010). Tomlinson et al. (2014) produced a model of the inattentive subtype of ADHD using the 5C-CPT by separating female Lister Hooded rats using parameters of above or below 90% accuracy with a SI of above or below 0.3, for high attentive and low attentive groups respectively across 5 baseline training sessions (Table 1). The SI adds an important factor to the model by accounting for vigilance, which has been shown to be a significant factor in all ADHD symptom subtypes (Collings, 2003). This therefore produces a model closer to the clinical situation, with the aim of improved assessment of compounds with differential efficacy in certain ADHD impulsive subtype patients.

2.3. Models most relevant to ADHD symptomatology

The earliest example of behavioural separation using the 5C-SRTT is by Puumala et al. (1996) (Table 1). Their method translates well to the combined subtype of ADHD. They produced the model by separating male Han:Wistar rats into an ADHD and a control group. To do this they used percentage correct responses and percentage premature responses. An important feature of their method is that they used two parameters to define the model, correctly recognising that ADHD commonly presents with both inattention and impulsivity, particularly in combined subtype patients (Epstein et al., 2003).

In an attempt to represent the spectrum of ADHD symptoms, Blondeau and Dellu-Hagedorn (2007) used a cluster analysis method to isolate an array of phenotypes (Fig. 3) (Table 1). This method produced the efficient performing (E) animals (high percentage correct, and low percentage premature), the moderate performing (M) group (with average percentage correct and percentage premature), the inattentive (IA) group (low percentage correct, but average percentage premature) and finally the inattentive and impulsive (IA-I) group (low percentage correct and high percentage premature). These groups are similar to those found in a clinical and non-clinical population when people were tested with a self-report questionnaire based on disease criteria (Marsh & Williams, 2004).

Tomlinson et al. (2015) also produced a model of adult ADHD combined subtype, this model used accuracy to separate by attentiveness, SI to separate by vigilance and pFA to separate by impulsive action (Table 1). In ADHD-C type patients these three symptoms combine to produce the most persistent and difficult to treat subgroup (Molina et al., 2009; Kooij et al., 2010). Therefore an animal model of this subtype is particularly valuable to enhance our understanding of the neurobiological basis of this subtype and so improve treatment strategies.

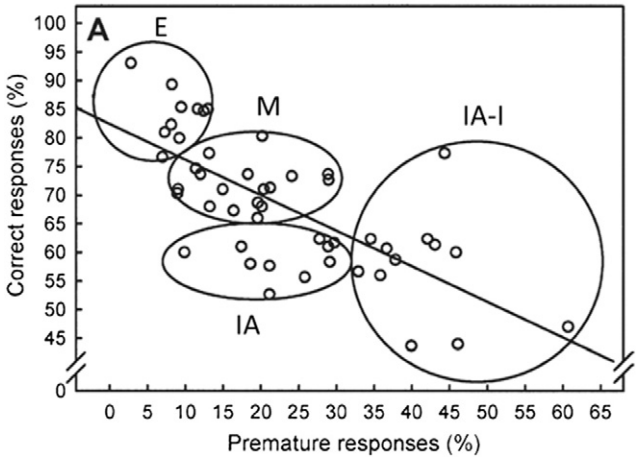

Fig. 3. A dimensional analysis method of performance separation. This method is particularly interesting as it produces multiple groups which correspond to clinical ADHD subgroups. Reproduced with permission, from Blondeau and Dellu-Hagedorn (2007).

3. Neurobiology identified using separation methods

3.1. Using impulsive models

3.1.1. Behaviour

Dalley et al. (2007) produced the most widely used behavioural separation model using the 5C-SRTT. It was proposed that differences in neurobiology between the animals in each group could drive escalated cocaine self-administration. Drug naive HI animals showed reduced binding potential for [^{18}F]fallypride using positron emission tomography imaging, suggesting that HI animals had a reduced number of D_2 and/or D_3 receptors in the ventral striatum, showing an endophenotype that may contribute to the behavioural differences. High impulsivity has been linked to reduced D_2/D_3 availability in the striatum of ADHD patients, which adds construct validity to the model (Buckholtz et al., 2010; Ghahremani et al., 2012). This difference was also observed following cocaine self-administration, which was enhanced in this group and correlated with reduced dopamine release in the nucleus accumbens core (NAcC) of HI animals. Diergaarde et al. (2008) used their model to show that high impulsive action predicted greater overall nicotine self-administration and longer maintenance compared with animals with lower impulsive action. They also showed that HI animals show escalated sucrose self-administration (Diergaarde et al., 2009). This shows that high impulsive animals are more responsive to reinforcement which is not limited to drugs of abuse. Dalley et al. (2007) showed that the HI phenotype is linked to increased cocaine self-administration. One caveat of previous findings is that in humans it was not possible to determine whether this high impulsivity is due to the drug taking itself or a predefined trait that led to escalating drug taking behaviour (Perry & Carroll, 2008). As impulsivity is screened before drug administration these findings support the latter theory. Belin et al. (2008) examined how HI animals differed in attempts to stop compulsive drug taking using the negative stimulus of a foot shock. They found that HI animals took more negative stimuli to stop drug taking behaviour showing that impulsivity is also linked to compulsive behaviour.

The delay discounting task challenges animals to decide how long they are willing to wait for a larger reward compared with a smaller and more immediate reward (Winstanley et al., 2003). This shows how impulsive the animals are, but is distinct from waiting impulsivity already discussed. This type of reward sensitivity is called impulsive choice, and is the ability to delay gratification for a better long term solution when faced with an immediate payoff. When HI animals in the Dalley et al. (2007) study were tested in the delay discounting task they showed a steeper discounting curve, showing that they are less willing to wait for a larger delayed reward as the delay increased (Robinson et al., 2009). However these rats showed no deficit in the stop signal reaction time task. This measures the speed at which an action can be cancelled. This highlights the separable nature of the different forms of impulsivity, which has previously been demonstrated in lesion studies (Cardinal et al., 2001; Eagle & Robbins, 2003; Christakou et al., 2004). Separation by performance using the delay discounting task has also been studied and is discussed in Jupp et al. (2013); here we focus on impulsive action in 5C-SRTT and 5C-CPT. Robinson et al. (2009) also demonstrated the ability of atomoxetine to reduce premature responses (waiting impulsivity) and flatten the delay discounting curve (impulsive choice).

3.1.2. Pharmacology/neurobiology

High waiting impulsivity in the Dalley et al. (2007) HI model can be modulated by dopaminergic and noradrenergic pharmacological agents such as quinpirole and sumanirole (both $D_{2/3}$ receptor agonists), which both reduce premature responses (Fernando et al., 2012) (Table 2). In that study, atomoxetine (a NA reuptake inhibitor) and guanfacine (an $\alpha 2$ adrenoceptor agonist) dose dependently reduced premature responses in HI animals. GBR-12909 (a DA reuptake inhibitor) increased impulsivity in HI and LI animals, whereas the DA and NA reuptake inhibitor methylphenidate had no effect on impulsive responses. These results show that both NA and DA systems are involved in impulsive behaviour and shows that activation of $D_{2/3}$ or $\alpha 2$ adrenoceptors is involved in impulse control. The modulation of impulsivity by these pharmacological agents is very similar to effects seen in humans with impulse control disorders and ADHD, demonstrating predictive validity of the model (Cunill et al., 2013; Wood et al., 2014). Glutamate is the most abundant excitatory neurotransmitter in the brain and shows involvement in a number of conditions which express high levels of impulsivity (Isherwood et al., 2015). Modulation of metabotropic glutamate receptors is therefore a target for investigation. Recent work examined the effect of metabotropic glutamate receptor 5 negative allosteric modulators RO4917523 and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) and the positive allosteric modulator ADX47273 on trait impulsivity (Isherwood et al., 2015) (Table 2). The authors demonstrated that RO4917523 and MTEP reduced premature responses but at the same time increased omissions and response latencies, suggesting a non-specific motor effect rather than a true reduction in waiting impulsivity. ADX47273 reduced premature responses in the HI group, where the effect was larger when animals were challenged with a 7 s rather than 5 s ITI. While in this group, omissions were not affected, correct latency was significantly increased at the same doses that reduced premature responses. A locomotor activity assessment was simultaneously conducted. The only dose of drug not to show any effect on locomotor activity was ADX47273 at 80 mg/kg, which also had a highly significant effect on correct latency. Due to these locomotor effects of the metabotropic glutamate receptor 5 modulators, it is challenging to separate effects on impulsivity from general effects on motor behaviour. Future work could investigate region specific infusion and compounds with affinity for other metabotropic receptors to analyse the role of glutamate in impulsivity without the confounding motor effects.

Based on the initial [^{18}F]fallypride PET study, the NAc, as part of the ventral striatum was found to be a key target, in particular, the $D_{2/3}$ receptor system (Dalley et al., 2007). The left NAc was found to have reduced grey matter density in HI animals (Caprioli et al., 2014). Infusion of aripiprazole, a $D_{2/3}$ receptor partial agonist and atypical antipsychotic, and nafadotride, a $D_{2/3}$ receptor antagonist, into the NAcC and nucleus accumbens shell (NAcS) separately showed a functional opposition within the NAc (Besson et al., 2010). For HI animals, nafadotride increased premature responses when infused into the NAcS, but reduced them when infused into the NAcC while aripiprazole had no effect when directly infused into either region (Table 2). This contrasting effect may explain why systemic administration of nafadotride had no effect on premature responses. This has been further investigated using quinpirole which was found to increase premature responses when infused into the NAcC, in HI animals only but increased locomotor activity in both HI and LI animals (Moreno et al., 2013). In contrast, infusion of quinpirole into the NAcS promoted an increase in locomotor activity only in HI and not in LI animals. Nafadotride did not attenuate the increase in impulsivity caused by quinpirole infused into the NAcC, however it did attenuate the locomotor stimulant effect of NAcS infusion in HI animals. The inhibitory neurotransmitter γ amino butyric acid (GABA) has recently been found to play a role in impulsivity by modulation in the NAcC. This was shown by a reduction of the enzymes needed to produce GABA, glutamate decarboxylase (GAD) 65/67, as well as reduced markers of dendritic spines and microtubules in HI compared to LI animals which was further strengthened by LI animals becoming more impulsive when GAD65/67 gene expression was reduced by infusion of antisense GAD67 and GAD65 oligonucleotides into the NAcC (Caprioli et al., 2014). This reveals a novel mechanism of impulsivity in rats and warrants further investigation.

Patients with damage to the orbital frontal cortex (OFC) have high scores on impulsiveness questionnaires (Berlin et al., 2005) and so Winstanley et al. (2010) investigated whether this region could be mediating behaviour in HI animals. OFC infusion of the dopamine D_1

Table 2
Summary of pharmacological effects in models based on behavioural separation. Abbreviations: Correct Latency (CL), noradrenaline (NA), NA transporter (NAT), Premature Responses (PR), dopamine transporter (DAT), catechol-O-methyl transferase (COMT) premature responses (PR), Nucleus accumbens (NAc), Na core (NAcC), Na shell (NAcS), metabotropic glutamate receptor 5 (mGluR5), prelimbic cortex (PrL).

	Model	Drug	Mechanism	Administration (route)	Measure	Effect (dose)	Notes	Reference
Impulsivity	Dalley et al. (2007) HI animals	Quinpirole	D2 agonist	Systemic (s.c.)	PR	↓ (0.01, 0.03, 0.1 mg/kg)		(Fernando et al., 2012)
		Sumanirole				↓ (0.1,0.3,1 mg/kg)	Also increased CL at 0.3 and 1 mg/kg	
		Atomoxetine	NAT inhibition	Systemic (i.p.)		↓ (1,3 mg/kg)	Also Increased CL at 3 mg/kg	
		Guanfacine	α2 NA Receptor agonist			↓ (0.3,1 mg/kg)		
		GBR 12909	DAT inhibition			↑ (2.5, 5 mg/kg)		
		Methylphenidate	DAT/NAT inhibition		–	–		
		Aripiprazole	D2/3 partial agonist	NAcC	–	–		(Besson et al., 2010)
				NAcS	–	–		
		Nafadotride	D2/3 antagonist	NAcC	PR	↓ (0.1 μg)		
				NAcS	PR	↑ (0.1 μg)		
		Quinpirole	D2/3 Full agonist	NAcC	PR	↑ (0.3, 1 μg)		(Moreno et al., 2013)
		RO4917523	mGluR5 negative allosteric modulator	Systemic (p.o.)	PR	↓ (0.1,0.3 mg/kg)	Increases in omissions, correct latency and locomotor activity	(Isherwood et al., 2015)
		MTEP		Systemic (i.p.)	PR	–	Increases in omissions, CL and locomotor activity, reduction in accuracy	
		ADX47273	mGluR5 positive allosteric modulator	Systemic (p.o.)	PR	↓ (40, 60, 80, 100 mg/kg)	Increases CL	
	Tomlinson et al. (2014) HI animals	Methylphenidate	DAT/NAT inhibition	Systemic (i.p.)	PR	↓ (0.5, 1 mg/kg)		(Tomlinson et al., 2014)
		Atomoxetine	NAT inhibition		PR	↓ (0.5, 1, 2 mg/kg)		
					pFA	↓ (1, 2 mg/kg)		
					SI	↑ (1, 2 mg/kg)		
Attention	Granon et al. (2000)	SCH 23390	D_1 antagonist	Bilateral PrL infusion	–	–		(Granon et al., 2000)
		SKF 38393	D_1 agonist		Accuracy	↑ (0.06 μg/side)		
					Omissions	↓ (0.06 μg/side)		
		Sulpiride	D_2 antagonist		–	–		
	Paterson et al. (2011)	D-Amphetamine	Catecholamine release	Systemic (i.p.)	PR Perservative responses	↑ (0.56, 1 mg/kg)		(Paterson et al., 2011)
		Methylphenidate	DAT/NAT inhibition		Omissions	↓ (0.1, 0.5 mg/kg)		
		Atomoxetine	NAT inhibition		PR	↓ (0.5,1,2 mg/kg)		
		Tolcapone	COMT inhibition		Omissions	↑ (3,30 mg/kg)		
	Grottick and Higgins (2000)	Nicotine	Nicotinic agonist	Subchronic systemic (s.c.) for 20 days	Accuracy	↑ (0.2 mg/kg/day)		(Grottick & Higgins, 2000)
					Correct latency	↓ (0.2 mg/kg/day)		
					PR	↑ (0.2 mg/kg/day)		
		SIB1765F	Nicotinic α4β2 agonist		Accuracy	↑ (5 mg/kg/day)		
					Correct latency	↓ (5 mg/kg/day)		
					PR	↑ (5 mg/kg/day)		
		AR-R 17779	Nicotinic α7 agonist		–	–		
	Tomlinson et al. (2014) HA animals	Methylphenidate	DAT/NAT inhibition	Systemic (i.p.)	Accuracy	↑ (2 mg/kg)		(Tomlinson et al., 2014)
					SI	↑ (0.5, 1 mg/kg)		
		Atomoxetine	NAT inhibition		Accuracy	↑ (2 mg/kg)		
Attention & impulsivity	Puumala et al. (1996)	Methylphenidate	DAT/NAT inhibition	Systemic (s.c.)	Accuracy	↑ (0.1, 1 mg/kg)		(Puumala et al., 1996)
	Blondeau and Dellu-Hagedorn (2007)	Methylphenidate	DAT/NAT inhibition		PR	↑ (1 mg/kg)	Largest effect in IA-I group	(Blondeau & Dellu-Hagedorn, 2007)
		Atomoxetine	NAT inhibition		PR	↑ (0.1, 0.5, 1 mg/kg)	In all but E group	
	Tomlinson et al. (2015)	A-412997	D4 agonist	Systemic (i.p.)	SI	↑ (0.3, 1 μmol/kg)		(Tomlinson et al., 2015)
					pFA	↓ (0.3, 1 μmol/kg)		
		Tolcapone	COMT inhibition		Accuracy	↑ (10, 15 mg/kg)		
					pFA	↓ (15 mg/kg)		
					SI	↑ (15 mg/kg)		

receptor antagonist, SCH23390 decreased impulsive responding in HI animals, but not in controls (Table 2). When infused into the OFC, quinpirole only affected non-impulsive parameters, while a D_1 receptor agonist, SKF-81297 had no effect. This shows that D_1 receptors in the OFC do not fully mediate effects on impulsivity suggesting that brain regions outside the OFC are important, and that D_2 receptors in the OFC are not involved in impulsivity. This study again demonstrated that baseline performance of the individual animal alters the effect of dopaminergic compounds.

Electrical signalling of clusters of neurons has also been linked to high and low impulsive behaviour (Donnelly et al., 2014). Silicone multielectrode arrays were implanted into the NAcC or NAcS and the infralimbic and prelimbic regions within the prefrontal cortex of HI and LI male Lister Hooded rats and low frequency (<200 Hz) activity recorded (Donnelly et al., 2014). By correlating electrical changes in the low frequency oscillations or local field potential with operant responses made in the 5C-SRTT, a new level of understanding was achieved. The authors discovered firstly that, across the NAc and prefrontal cortex, gamma (50–60 Hz) and theta (7–9 Hz) frequency oscillations increased during visual search in anticipation of stimulus presentation and again following a correct response. Secondly they found that gamma and delta activity coupling strengthened following error responses. Thirdly they found that these differed significantly when a premature response was about to be made. Finally they found that in HI animals error responses were stronger, and responses linked to correct responses were weaker. This elegant work shows that both the medial prefrontal cortex and the NAc are important regions for attention when scanning for a target and are linked to impulsive responses by recognition of error response. This series of experiments shows that, not only can behavioural separation offer a platform to test and compare future treatments, but enable research into new mechanisms which cause behavioural deficits. Other models such as dopaminergic lesion would not have allowed insight into the GABAergic or electrical activity within discrete brain areas which are becoming of increasing relevance in the search for alternative treatment mechanisms (Jupp et al., 2013; Donnelly et al., 2014; Hayes et al., 2014).

3.2. Using models of attention

Granon et al. (2000) used the parameters of above or below 75% accuracy to analyse the effect of three dopaminergic compounds via bilateral microinfusion into the medial prefrontal cortex. They found that, while sulpiride (a D_2 receptor antagonist) had no effect on task performance, SKF-38393 (a D_1 receptor agonist) enhanced accuracy selectively in the low performing animals and SCH 23390 (a D_1 receptor antagonist) had the opposite effect to reduce accuracy selectively in the high performing animals (Table 2). Although SKF-38393 had no effect here in unseparated animals, it may be due to the task used, as it has an effect on vigilance in 5C-CPT (Barnes et al., 2012a). The selectivity of effect suggests that there could be differences in dopamine system engagement in high and low performing animals, where low performing animals have reduced dopamine activity in the prefrontal cortex making them susceptible to receptor activation and high performing animals have increased levels of dopamine and are thus less susceptible to receptor activation, but more susceptible to antagonism.

Work by Paterson et al. (2011) expanded this by also analysing effects of d-amphetamine, methylphenidate and atomoxetine in poor performing animals. They used similar methods of separation to Granon et al. (2000), but only studied poor performing animals. They also compared the effect of these clinically efficacious compounds to tolcapone (a Catechol O Methyl Transferase (COMT) inhibitor). They found that a systemically administered acute dose of d-amphetamine increased premature responding in the poor performing rats. Atomoxetine reduced the number of premature responses. Methylphenidate reduced percentage omissions at low doses (0.1 and 0.5 mg/kg) (Table 2). One issue with this study, however, is the use of only the poor performing animals and not both poor and high performing animals which would allow a more robust comparison. In ADHD (discussed later) an oppositional effect is seen where ADHD-like animals show improved performance and good performers show reduced performance in response to methylphenidate.

Using low performing animals, Grottick and Higgins (2000) found that sub-chronic dosing of nicotine and SIB 1765 F, an $\alpha4\beta2$ nicotinic acetylcholine receptor agonist, improved accuracy and speed of response, whereas AR-R 17779, an $\alpha7$ nicotinic receptor agonist, had no effect (Table 2). Nicotine and SIB 1765 F both increased premature responding during sub-chronic treatment, while nicotine also reduced omissions. This shows that nicotine and SIB 1765 F improve selective attention, and speed of response processing but increase waiting impulsivity, suggesting that the effects of nicotine are mediated via the $\alpha4\beta2$ nicotinic acetylcholine receptor and not the $\alpha7$ receptor. The effect of nicotine on omissions may be the result of an effect on motor activity (Grottick et al., 2003; Young et al., 2013). It has since been found in unseparated mice and in mice with a scopolamine (muscarinic acetylcholine receptor agonist) induced vigilance deficit that nicotine has a primary effect on vigilance (Young et al., 2013). This would make it an interesting pharmacological agent to test using animals separated in the 5C-CPT due to the ability to robustly calculate vigilance in that task.

3.3. Using models of ADHD

Lower right frontal cortex DOPAC/DA ratio has been observed in animals with lower accuracy, whereas in the left frontal cortex serotonin levels were higher in animals with low accuracy (Puumala & Sirvio, 1998). Furthermore, serotonin utilization in the right frontal cortex was higher in highly impulsive animals. This suggests that pharmacological agents developed to improve poor performance should increase dopaminergic neurotransmission and reduce serotonergic neurotransmission in the frontal cortex. Methylphenidate (MPH) increases DA and NA by blocking the dopamine transporter (DAT) and noradrenaline transporter thus reducing reuptake of DA and NA leading to increased activation of D_1 and $\alpha2$ receptors respectively. This has a positive effect on choice accuracy in low attentive animals (Puumala et al., 1996; Blondeau & Dellu-Hagedorn, 2007). Doses of 100 and 1000 μg/kg MPH were used in the 5C-SRTT, the lower dose also reduced premature responses in low performing animals (Table 2). However it has also been found that MPH can increase premature responding at high doses of 1 mg/kg (Blondeau & Dellu-Hagedorn, 2007). The differential effect of methylphenidate in high and low attentive animals is interesting as it can be understood using the inverted U shaped hypothesis of optimal arousal, where alertness can only be increased to a point, beyond which further stimulation of the system promotes negative effects such as distractibility, impulsiveness and anxiety (Wood et al., 2014). A neurobiological theory of ADHD has been suggested based on this, where prefrontal cortex catecholamine levels follow this inverted U shaped relationship (de Jongh et al., 2008; Arnsten, 2009). This also shows that separation of the animals is essential to determine doses of compounds which may selectively improve the performance of low and high performing animals. Future work should aim to establish the mechanisms behind the difference in sensitivity to monoaminergic compounds.

Atomoxetine is a second line treatment for ADHD and acts in a similar way to methylphenidate, but is selective for NAT, with minimal affinity for DAT, thus activating $\alpha2$ adrenoceptors in the prefrontal cortex and minimising striatal psychostimulant effects (Bymaster et al., 2002; Heal et al., 2009). In animals separated by dimensional analysis into efficient (E), moderate (M), Inattentive (IA) and Inattentive and Impulsive (IA-I) groups, atomoxetine had no effect on accuracy, but reduced premature responses in all groups except E, at doses of 0.1, 0.5 and 1 mg/kg (Table 2). This suggests that atomoxetine requires a certain threshold of premature responding to have a beneficial effect (Blondeau & Dellu Hagedorn, 2007).

3.4. Using models developed with the 5C-CPT

A selective effect of MPH has also been shown in the 5C-CPT in female Lister Hooded rats. Accuracy increased in the LA animals (representing the ADHD-I subtype) at 2 mg/kg, while SI increased only at 0.5 and 1 mg/kg (Tomlinson et al., 2014) (Table 2). Firstly, this suggests a pharmacological separation between selective attention and vigilance, secondly, the lack of effect on vigilance at 2.0 mg/kg may again be explained by the inverted U shaped hypothesis of optimal arousal (Wood et al., 2014). Furthermore in LI animals, MPH increased premature responses at the highest dose of 2 mg/kg. In animals with high levels of premature responding and pFA (HI), MPH reduced premature responses at 0.5 and 1 mg/kg. As no effect on pFA was observed at any dose of MPH, this can be seen as an effect on waiting impulsivity and not response disinhibition.

In the same study, atomoxetine increased accuracy at 2 mg/kg and SI at 1 and 2 mg/kg in LA animals, but reduced accuracy in HA animals at 1 mg/kg (Tomlinson et al., 2014). This adds to Blondeau & Dellu-Hagedorn's study (2007) showing no effect up to 1 mg/kg for accuracy in their model using the 5C-SRTT and for the first time uses a robust calculation of vigilance which is increased by atomoxetine. Atomoxetine also reduced premature responding in HI animals at all doses and reduced pFA in HI (1 and 2 mg/kg) and LI (2 mg/kg) animals. This differential effect of atomoxetine further emphasises the need to separate animals based on their performance in order to clearly identify effects of pharmacological agents.

Tomlinson et al. (2015) produced a low attentive, low vigilance and high response disinhibition model of ADHD-C using female Lister Hooded rats in the 5C-CPT. A-412997, a selective DRD4 receptor agonist improved vigilance and reduced pFA at 0.3 and 1 μmol/kg (Table 2). No effect was seen on any parameter in the HA animals, suggesting that targeting the DRD4 receptor could be an effective treatment for the combined subtype of ADHD. This is a particularly important finding as a genetic mutation of the 7 transmembrane repeat of DRD4 is linked to adult ADHD, (Franke et al., 2012), and several D4 receptor antagonists have improved behavioural hyperactivity in an ADHD animal model (Zhang et al., 2002), highlighting the importance of this receptor system in ADHD pathophysiology.

The COMT inhibitor, tolcapone has previously been shown to be ineffective in the 5C-SRTT (Paterson et al., 2011). The previous method of separation was based only on accuracy and used the 5C-SRTT, whereas Tomlinson et al. (2015) used tolcapone in the ADHD-C model, in the 5C-CPT. In that study, accuracy and SI improved in ADHD-C animals (at 10 and 15 mg/kg), but decreased in HA animals at the highest dose of 15 mg/kg. There are several possible explanations for this discrepancy; firstly the 5C-CPT compared to the 5C-SRTT is a more difficult task, whereby the extra difficulty may have challenged the animals enough to see an effect (Lustig et al., 2013). Secondly, the Tomlinson et al. (2015) method used a particularly rigorous model of ADHD subtype ADHD-C, this involved the two extra parameters of SI and pFA and a higher level of accuracy, resulting in a different model. This highlights the potential flexibility of separation by performance to produce two very different models depending on the parameters used for separation. It is important that the most appropriate method of separation is used depending on the experimental question being investigated. This subject is addressed below.

4. Selection of the most appropriate method of separation

When deciding which model is best, the first question is, what is being modelled. As shown throughout this review modelling by performance is flexible allowing many measures to be used individually or collectively. This decision also dictates which task to use. For example for a model concerning deficits in vigilance, as seen in ADHD or schizophrenia, then the 5C-CPT is the most viable choice. Also, models involving impulsive action should strongly consider the important difference between response disinhibition and waiting impulsivity (discussed above) as these have been shown to be separate constructs, which can be manipulated independently (discussed in Young et al. (2011)). However, for models of selective or sustained attention, the 5C-SRTT measures of accuracy and omissions are well established parameters. The reduced length of training time in the 5C-SRTT compared to the 5C-CPT can also be a determining factor in choice of test.

Separation method differences are clear from the studies outlined in this review. To decide between these factors there are a series of questions to ask. What level of deficit is required? Winstanley et al. (2010) produced a model with a milder impairment using a simple median split. In contrast, upper and lower quartile separation used by Diergaarde et al. (2008) removes a middle performing group to produce a model with a larger deficit. A larger deficit can also be produced by increasing the challenge in the task. Dalley et al. (2007) provide an excellent example of introducing an additional challenge in the form of longer ITIs to further separate the animals, producing a larger deficit as shown by their model producing the highest 15th percentile of the group (Isherwood et al., 2015). When choosing a more rigorous method it is important to consider the principles of the 3Rs: replacement, refinement and reduction. A larger starting cohort will be required to maintain statistical power in the group selected as a model. This contradicts the principle of reduction of number of animals used, as suggested by the national centre for the replacement, refinement and reduction of animals in research, NC3Rs, so the two must be carefully balanced.

Methods of comparison vary between studies; a key factor to consider is within study referencing compared to between study referencing. A within study reference is the more common approach. This involves using measures such as mean, median, quartiles or percentiles to use the group's performance to determine which animals are high versus low performers within that group. A main advantage of this method includes consistent group numbers in high or low groups, making comparisons more robust. Also it provides the opportunity for translation of this modelling method between strain, gender or species which may have different baseline levels of performance. If the groups differ in baseline performance then this too is a confounding factor. To account for this, whole group baseline performance is important to consider when comparing between studies using this method, and the separation levels should be clearly stated.

In between study referencing, a line is produced from the data (e.g., <75%) and is then used in that study and for future studies. This method provides more robust comparison between experiments. However, how these reference lines are determined is often not clearly explained, questioning the validity of this approach. Finally, a very interesting unique method is the dimensional analysis used by (Blondeau & Dellu-Hagedorn, 2007). This method allows an unbiased approach to selecting groups with different profiles using multiple measurements. As they have demonstrated, this allows a range of profiles to be produced within a population. The use of additional measures such as those in the 5C-CPT would allow new types of models to emerge from this method.

5. Conclusion

Animal models have several advantages including accurate control of variables and manipulation of subjects. Recent models have attempted to recreate the symptomatology of disorders characterised by attention and impulsivity deficits, using chemical lesions or genetic manipulations. In a diverse population, where the aetiology is unknown, these methods restrict the ability to identify novel targets for drug discovery. Separating animals based on performance in 5C-SRTT and 5C-CPT offers a method of modelling without the limitation in assuming a mechanism of action. Behavioural separation offers the further advantage of enhanced flexibility to investigate many conditions which are characterised by low levels

of attention or high impulsivity such as ADHD, drug abuse, obsessive compulsive disorder, Parkinson's disease and schizophrenia.

Through continued use of these models, mechanisms underlying impulsivity have been identified such as fronto-striatal interaction and the involvement of GABAergic signalling within the NAc. The translational validity of these methods has been clearly demonstrated, particularly in relation to the HI model of Dalley et al. (2007). It is hoped that many of the other models will be used to the same level in order to determine reliability and improve translation. The series of models recently produced using the 5C-CPT strive to improve translation of this work into a clinical setting by mimicking more symptoms and offering specialised models for patient subtypes (Tomlinson et al., 2014, 2015). This aims to move towards a more individualised treatment method where compounds can be pre-clinically tested for a variety of symptom subtypes. Some progress has already been made in this area for the standard ADHD treatments, MPH and atomoxetine. Separation of differential drug effects in animals separated by performance corresponds with the inverted U shaped hypothesis of optimal arousal. Indeed this hypothesis highlights why behavioural separation is so important. Pharmacological treatments clearly act differently in animal subjects with poor attention or impulsivity and so need to be studied in these clinical populations. Finally, one of the major considerations in this area is determination of the neurobiological basis of these differences in performance in preclinical models and clinical populations.

Conflicts of interest statement

JCN has received honoraria for lecturing and consultancy work and serves on the advisory boards of several pharmaceutical companies.

Funding sources

AH is a PhD student part-funded by the University of Manchester (UoM) Pharmacy School and b-neuro at the UoM. JCN is a full-time employee of the UoM.

Acknowledgments

The authors would like to thank Jeff Dalley for his constructive comments on the manuscript.

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