Investigation of the relationships between rat ultrasonic vocalizations, reward and dopamine transmission

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ABSTRACT

Adult rats emit ultrasonic vocalizations primarily around two frequencies: 22- and 50-kHz. These two broad categories of calls are commonly associated with contexts and stimuli that are either aversive or reinforcing, respectively. The 50-kHz call type can be subdivided into 14 subtypes, and several lines of evidence suggest that the mesolimbic dopamine (DA) system promotes 50-kHz call emission.

The first aim of this thesis (Chapter 2) was to determine whether 50-kHz calls can be elicited by enhancing DAergic signaling pharmacologically. Instead, systemic administration of selective DAergic receptor agonists decreased 50-kHz call rates, thus mimicking the effects of DA receptor antagonists. These findings suggested that DAergic transmission is necessary, but is not sufficient, for call emission.

According to recent reports, psychostimulants (e.g. cocaine and amphetamine), which reliably increase 50-kHz calls, also increase *phasic* DA release. To reconcile the seemingly conflicting findings from Chapter 2, we hypothesized that *phasic* DA release is required for the production of 50-kHz calls. According to this hypothesis, tonic activation of postsynaptic receptors by DAergic agonists would have inhibited the transmission of phasic DA signals, thereby producing a call-depressant effect, as observed following the administration of antagonists. Therefore, in Chapter 3, we investigated the relationship between *phasic* DA release and 50-kHz vocalizations. First, we confirmed that electrical stimulation of the medial forebrain bundle (MFB) produced 50-kHz vocalizations. We showed that these calls were time-locked with phasic DA release, which we recorded in the nucleus accumbens (NAcc) of freely moving animals using fast-scan

cyclic voltammetry (FSCV). The associated call profile, with a preponderance of trill calls, was similar to that following administration of psychostimulants. Next, we showed that optogenetic stimulation of ventral tegmental area (VTA) DAergic neurons was also associated with high call rates, with trills again predominating. Call rates were significantly higher during non-contingent *vs.* response-contingent stimulation. Although rats continued to respond for, or passively receive, optogenetic stimulation throughout the 2-hour session, they virtually ceased calling within 30 minutes. Taken together, the results from Chapter 3 suggest that phasic DA release in the mesolimbic system, even though reinforcing, is not sufficient to elicit 50-kHz calls.

In a final study (Chapter 4), we investigated the relationship between phasic DA release and *amphetamine (AMPH)-induced* calls. Systemic administration of AMPH increased the 50-kHz call rate, with an inverted-U shaped dose-response relationship. Conversely, co-administration of selective DAergic agonists with AMPH significantly decreased calls. Next, we recorded phasic release in the NAcc using FSCV following the acute administration of AMPH. AMPH (tested at 0.3 - 4 mg/kg) also increased the amplitude of DA transients without affecting their duration or frequency. As expected, AMPH also significantly increased 50-kHz call rates. However, phasic DA release in the NAcc did not co-occur with 50-kHz calls. Furthermore, there was no correlation between the number of USVs and the frequency, duration or amplitude of transients, suggesting that phasic DA release in the NAcc is not necessary for the emission of AMPH-induced vocalizations.

In summary, the present findings indicate that although 50-kHz call emission is strongly dependent on DA transmission, phasic DA release in the NAcc appears neither sufficient nor necessary for their occurrence.

RESUMÉ

Les rats adultes émettent des vocalisations dans la fréquence ultrasonique, principalement à 22 et 50 kHz. Ces deux catégories d'appels sont associées à des contextes soit d'aversion ou de récompense, respectivement. La catégorie de vocalisation à 50 kHz peut être subdivisée en 14 sous-catégories. De plus, plusieurs découvertes supportent un rôle pour le système mésolimbique de dopamine pour la production de ces appels.

Le premier objectif de cette thèse (Chapitre 2) était de déterminer si l'activation de signalisation dopaminergique serait suffisante pour produire des appels à 50 kHz. Plutôt qu'augmenter les appels, les injections systémiques d'agonistes dopaminergiques sélectives ont *diminué* la production d'appels à 50 kHz, ainsi reproduisant l'effet des antagonistes. Ces résultats suggèrent que l'activation de signalisation dopaminergique est nécessaire mais n'est pas suffisante pour produire des appels de 50 kHz.

Selon des rapports récents, les psychostimulants (ex: cocaïne et amphétamine), qui produisent des appels de 50 kHz de manière fiable, produisent aussi la libération phasique de dopamine. Donc, afin de réconcilier les résultats apparemment contradictoires du Chapitre 2, nous avons formulé l'hypothèse suivante: la libération *phasique* de dopamine est requise pour la production d'appels de 50 kHz. Selon cette hypothèse, l'activation tonique de récepteurs post-synaptiques par des agonistes dopaminergiques aurait empêché la transmission de signal phasique, produisant alors une inhibition d'appels, tel qu'on a observé avec les antagonistes. Donc, dans le Chapitre 3, nous avons étudié la relation entre la libération phasique de dopamine et les vocalisations de 50 kHz. En premier, nous avons confirmé que la stimulation électrique du faisceau médian du

télencéphale produit des appels de 50 kHz. Nous avons démontré que ces appels se produisent en même temps que la libération phasique de dopamine dans le noyau accumbens, que nous avons mesurée en utilisant la voltammétrie cyclique ultra rapide. Le profil d'appels, avec une prépondérance de la sous-catégorie de 'trill', était semblable à ce que l'on observe suite à l'administration d'amphétamine. Par la suite, nous avons montré que la stimulation optogénétique de neurones dopaminergiques de l'aire tegmentale ventrale était associée avec un taux élevé d'appels de 50 kHz, avec encore une fois, une prépondérance de 'trill'. Le taux d'émission d'appels était significativement plus élevé lors de la stimulation sans contingence vs. la stimulation avec contingence. Même que les rats ont travaillé pour recevoir ou ont reçu passivement la stimulation optogénétique pendant la session de 2 heures, ils ont cessé d'émettre des vocalisations ultrasoniques dès 30 minutes. Pris dans leur ensemble, les résultats du Chapitre 3 suggèrent que la libération phasique de dopamine dans le système mésolimbique, même que gratifiante, n'est pas suffisante pour provoquer des appels de 50 kHz.

Dans une dernière étude (Chapitre 4), nous avons investigué la relation entre la libération phasique de dopamine et les appels provoqués par l'amphétamine. L'administration systémique d'amphétamine à augmenter le taux d'appels de 50 kHz, décrit par une courbe dose-réponse en U-inversé. Contrairement, la co-administration d'agonistes dopaminergiques sélectives avec l'amphétamine, a diminué le taux d'appels significativement. Par la suite, nous avons mesuré la libération phasique de dopamine dans le noyau accumbens en utilisant la voltammétrie cyclique. Amphétamine (testé à 0.3-4 mg/kg) a aussi augmenté l'amplitude de dopamine sans affecter la durée ni la fréquence. Comme prévu, amphétamine a augmenté l'émission d'appels de 50 kHz. Par contre, la libération phasique de dopamine ne s'est pas produit en même temps que les

appels. De plus, il n'y avait aucune corrélation entre le nombre d'appels ultrasoniques et la fréquence, durée ou amplitude de dopamine; ceci suggère que la libération phasique de dopamine dans le noyau accumbens n'est pas nécessaire pour la production d'appels de 50 kHz provoquée par l'amphétamine.

En résumé, les résultats de cette thèse indiquent que quoique la production d'appels de 50 kHz dépend fortement sur la transmission dopaminergique, la libération phasique de dopamine dans le noyau accumbens ne semble pas être suffisante ni nécessaire pour leur production.

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CONTRIBUTION OF AUTHORS

This manuscript-based thesis is composed of three manuscripts. Author contributions are as follows.

Chapter 2: Scardochio T, Clarke PBS (2013) Inhibition of 50-kHz ultrasonic vocalizations by dopamine receptor subtype-selective agonists and antagonists in adult rats. Psychopharmacology (Berl) 223(6):589-600

All of the experimental work was performed by Tina Scardochio. The first draft and all subsequent manuscript drafts were written by Tina Scardochio and revised with Dr. Paul Clarke.

Chapter 3: Scardochio T, Trujillo-Pisanty I, Shizgal P, Clarke PBS (in preparation) Investigation of the role of phasic dopamine release in rat ultrasonic vocalizations: insights from electrical brain stimulation and optogenetics

All of the experimental work was performed by Tina Scardochio with the following exceptions: optogenetic-related surgical operations and histological verification of optogenetic virus were performed by Ivan Trujillo-Pisanty. Supporting data (part of supplementary Fig. 5) were obtained by Marie-Pierre Cossette and Dr. Kent Conover helped with statistical analysis. To date, the first draft and all subsequent manuscript drafts were written by Tina Scardochio and revised with Dr. Paul Clarke. Subsequent drafts will be revised with Dr. Peter Shizgal and Ivan Trujillo-Pisanty.

Chapter 4: Scardochio T, Sandberg SG, Phillips PE, Clarke PBS (in preparation) Investigation of the role of phasic dopamine release in amphetamine-induced rat ultrasonic vocalizations

All of the experimental work was performed by Tina Scardochio. Dr. Stefan Sandberg provided training and guidance for the fast-scan cyclic voltammetry technique. To date, the first draft and all subsequent manuscript drafts were written by Tina Scardochio and revised with Dr. Paul Clarke. Subsequent drafts will be revised with Dr. Paul Phillips and Dr. Stefan Sandberg.

CONTRIBUTIONS TO ORIGINAL KNOWLEDGE

Chapter 2:

- Spontaneous 50-kHz ultrasonic vocalizations (USVs) decreased following systemic injection of the following drugs: selective D1-like and D3-selective DAergic antagonists; selective D1-like, D2/D3- and D3- agonists; combinations of D1-like and D2/D3 agonists; and agonist-antagonist combinations. In contrast, a D4 agonist and antagonist did not significantly affect 50-kHz call rate.

Chapter 3:

- Unexpected electrical stimulation of the medial forebrain bundle (MFB) elicited both 50-kHz USVs and phasic dopamine (DA) release events in the nucleus accumbens (NAcc). The onset of calls was time locked with transient DA release. Most 50-kHz calls emitted following electrical stimulation of the MFB were of the 'trill' call subtype
- During optogenetic stimulation of ventral tegmental area (VTA) dopaminergic (DAergic) neurons, the USV call rate and call profile were similar to that observed following the systemic administration of psychostimulants
- Rats decreased their call rate over the first 30 minutes of the session, despite near-constant rates of responding for optogenetic stimulation of VTA DAergic neurons. This disparity occurred with each of the four different schedules of stimulation delivery (fixed-interval 20s, fixed-time 20s, variable-interval 20s, variable-time 20s)
- Rats emitted significantly more calls during sessions providing non-contingent optogenetic stimulation than during sessions offering contingent stimulation

- Rats did not appear to call in anticipation of optical stimulation, and the 50-kHz call profile was comparable before and after optical stimulation

Chapter 4:

- The dose-response relationship between amphetamine (AMPH; 0.1, 0.3, 0.6, 1.6 and 4 mg/kg) and 50-kHz vocalizations followed an inverted-U shape where AMPH increased 50-kHz call rate at the three highest doses
- AMPH-induced 50-kHz vocalizations decreased following co-administration with D1-like and D2/D3 DA receptor agonists
- AMPH (0.3, 0.6, 1.6 and 4 mg/kg) significantly increased the amplitude of phasic DA release events in the NAcc, without detectably altering their frequency of occurrence or duration
- The majority (>95%) of USVs did not co-occur with phasic DA release in the NAcc

LIST OF ABBREVIATIONS

5-HT	serotonin
AMPH	amphetamine
ChR2	channelrhodopsin-2
CPA	conditioned place aversion
CPP	conditioned place preference
CTL	control
CRE	Cre recombinase
CV	cyclic voltammogram
DA	dopamine
DAergic	dopaminergic
DAT	dopamine transporter
DMSO	dimethyl sulfoxide
FSCV	fast-scan cyclic voltammetry
GLUT	glutamate
ICSS	intra-cranial self-stimulation
IP	intraperitoneal
MDMA	3,4-methylendioxy-methamphetamine
MFB	medial forebrain bundle
NA	noradrenaline
NAcc	nucleus accumbens
NAergic	noradrenergic
SAL	saline

SC	subcutaneous
TH	tyrosine hydroxylase
TH:Cre	tyrosine hydroxylase: Cre recombinase
USV	ultrasonic vocalization
VEH	vehicle
VTA	ventral tegmental area

CHAPTER 1: Introduction and Literature Review

Tina Scardochio

General introduction

Mental health disorders such as schizophrenia, depression and substance abuse are a major public health concern that impacts all societies on many levels; either directly or indirectly, everyone experiences the repercussions. An estimated 1 in 5 Canadians experiences a mental health or addiction problem in a given year (Kirby and Keon 2004) which translates to roughly 6.8 million people; by comparison, 2.2 million people in Canada have type 2 Diabetes (Statistics Canada 2014). In addition to the serious health consequences, the economic and social burden is tremendous. Mental health problems cost our health care system $50 billion per year; this represents almost 3% of Canada's gross domestic product (Mental Health Commission of Canada 2011). The repercussions of untreated mental health disorders include intoxicated driving (Freeman et al. 2011), violence (Werb et al. 2011), stress (Hammen 2005; Bardo et al. 2013) and suicide (Miller et al. 2014; Carra et al. 2014). Currently there are no cures for most mental health problems; conventional therapies only help to *manage* the disorders (Pouget and Muller 2014; Newby et al. 2015). In substance abuse, where drug treatments show promise in initial phases of rehabilitation, the relapse rates are comparable to other chronic illnesses such as hypertension, Type I diabetes and asthma (McLellan et al. 2000).

Due mostly to ethical constraints, the scope of human studies is limited, with a strong emphasis on non-invasive approaches such as brain imaging (Daglish and Nutt 2003; Phillips et al. 2015). For this reason, animal models of mental illness have been developed to try to mimic the many behavioural and physiological manifestations (Lynch et al. 2010; Jones et al. 2011; Planeta 2013; Hollis and Kabbaj 2014). For example, (1) conditioned place preference (CPP) is used to assess the rewarding effects of a variety of drugs (Tzschentke 2007). Here, animals spend more time in

an environment previously paired with a rewarding stimulus. (2) In the forced-swim test, a behavioural screen for antidepressant drugs, animals are placed in a cylinder filled with water. Subjects then become immobile when faced with the inescapable nature of the apparatus (Bogdanova et al. 2013). One main drawback of almost all existing behavioural tests is that the subjective state of the animal cannot readily be inferred. To provide a more direct measure of affect for the improvement of animal models, more recently studies have focused on decoding animal communication and language (Takahashi et al. 2010; Brainard and Fitch 2014; Kershenbaum et al. 2014; Kriengwatana et al. 2014). The main goal for improving these animal models is to have a better understanding of the complex psychosocial and neurobehavioural components of mental health disorders, to ultimately create better treatments.

An introduction to rat ultrasonic vocalizations (USVs)

In the early 1940s, it was discovered that rats could hear in the ultrasonic range; this was first demonstrated in rats that learnt to react to ultrasound signals thereby avoiding footshock (Gould and Morgan 1941). More than a decade later, Anderson (Anderson 1954) reported that rats can vocalize in the ultrasonic range, for example in response to an aversive tail pinch. Since then, two main types of adult rat USVs have been categorized according to their mean frequencies: 22-kHz (range: 18-28 kHz) and 50-kHz calls (30-90 kHz) (Portfors 2007; Wohr and Schwarting 2013; Clarke and Wright 2015). These two call categories not only occupy separate frequency ranges, but differ along several other acoustic dimensions. Notably, 50-kHz calls are much softer and briefer (~0.05 ms vs. ~0.5-2 s), and tend to be more frequency modulated (Blanchard et al. 1992; Brudzynski 2005). The 22-kHz calls are commonly produced in aversive contexts: drug withdrawal (Vivian and Miczek 1991; Covington, III and Miczek 2003; Berger et al. 2013),

stress (Knapp and Pohorecky 1995; Inagaki et al. 2005; Yee et al. 2012), predatory exposure (Blanchard et al. 1991; Blanchard et al., 1992), inter-male aggression (Sales 1972; Corrigan and Flannelly 1979; Thomas et al. 1983), electric shock (van der Poel et al. 1989; Kaltwasser 1990; Parsana et al. 2012) and after an unexpected decrease in reward size (Coffey et al. 2012). Taken together, the main function of these 22-kHz calls appears to be in signalling alarm and conveying negative affect and anxiety. In line with this, the production of these calls has been linked to brain regions such at the amygdala, perirhinal cortex and periaqueductal gray which play a role in, among other functions, fear conditioning (Allen et al. 2007; Sadananda et al. 2008; Wohr and Schwarting, 2013; Kim et al. 2013; McCue et al. 2014).

Adult rat 50-kHz vocalizations, in contrast, are commonly observed in association with appetitive or reinforcing stimuli (Burgdorf et al. 2011; Seffer et al. 2014). For example, 50-kHz USVs have been observed during social contact or cooperative behaviour (Brudzynski and Pniak 2002; Lopuch and Popik 2011), and rough-and-tumble play (Knutson et al. 1998), and following AMPH administration (Burgdorf et al. 2001; Wintink and Brudzynski 2001; Thompson et al. 2006; Ahrens et al. 2009; Wright et al. 2010; Simola et al. 2012; Wright et al. 2013; Ahrens et al. 2013; Chapter 2: Scardochio and Clarke 2013) and reinforcing electrical brain stimulation (Burgdorf et al. 2000; Burgdorf et al. 2007). Recently, 50-kHz calls have been proposed as an index of incentive salience (Brenes and Schwarting 2014). More specifically, sign-tracking rats emitted more 50-kHz calls than goal-trackers (Brenes and Schwarting 2015).

Commonly, 50-kHz calls are pooled into two categories, where frequency modulation is the sole determining factor (flat vs. frequency-modulated) (Ciucci et al. 2009; Popik et al. 2014; Opiol et

al. 2015). However, the 50-kHz category can be subdivided into *14* subtypes based on several acoustic parameters including duration and frequency modulation (Wright et al., 2010). Only in the past 3 years have researchers begun incorporating our 14 subtypes, the most detailed categorization to date, into their analysis (Simola et al., 2012; Himmler et al. 2014b). Recent studies have shown that the prevalence of certain call subtypes is context-dependent. For example, the proportion of flat calls increases upon separation from a cage mate (Wohr et al. 2008), during evasion in play behaviour (Takahashi et al., 2010) and following reunion of a dam with her pups (Stevenson et al. 2009). The flat call type is also more prevalent in rats possessing the novelty- and sensation-seeking phenotype (Garcia et al. 2015). In contrast, the trill subtype is more frequently associated with appetitive contexts such as play behaviour (Himmler et al. 2014a) and receipt of drug rewards such as psychostimulants (Wright et al., 2013; Ahrens et al., 2013; Chapter2: Scardochio and Clarke, 2013; Barker et al. 2014b).

Briefly, USVs potentially reflect a 'rat language' that is as yet largely unexplored. The acoustic and behavioural complexities of 50-kHz calls may provide important information that can be harnessed to create more robust and informative animal models of neuropsychiatric conditions.

Associations of 50-kHz USVs with affect

The occurrence of 50-kHz calls in appetitive situations has led to the proposal that the 50-kHz call rate provides a measure of positive affective state. Although we cannot explicitly know whether positive affect exists in rats, many researchers in the USV field assume this to be true (Panksepp and Burgdorf 2000; Knutson et al. 2002; Panksepp and Burgdorf 2003; Covington, III and Miczek, 2003; Brudzynski 2007; Stevenson et al., 2009; Burgdorf et al. 2009; Mallo et al.

2009; Meyer et al. 2011; Browning et al. 2011; Burgdorf et al., 2011; Rygula et al. 2012; Wohr and Schwarting, 2013; Berger et al., 2013; Pereira et al. 2014; Binkley et al. 2014; Kisko et al. 2015). In this thesis, we are assuming that rats experience affect. In the following section, I first describe the main lines of supportive evidence for a role of 50-kHz calls in affect, and in a subsequent section, I will discuss evidence that challenges this hypothesis. First, then, the supportive evidence based on 50-kHz call rate:

(1) Both amphetamine and cocaine reliably elicit 50-kHz calls (Ahrens et al., 2009; Wright et al., 2010; Ma et al. 2010; Wright et al. 2012b; Simola et al. 2014). Moreover, rats that produced more calls in response to an acute amphetamine injection also produced a larger CPP, a validated measure of reward (Ahrens et al., 2013). Similarly, rats that attribute greater incentive salience to a reward cue also produce more 50-kHz vocalizations when injected with cocaine or when exposed to a cocaine cue (Meyer et al., 2011).

(2) Alcohol dependent rats increased their 50-kHz call rate when exposed to an alcohol-associated context (Buck et al. 2014a). In addition, both dependent and non-dependent rats increased their 50-kHz call rate in anticipation of alcohol availability. Collectively, these findings suggest that 50-kHz USVs may be a reliable predictor of the motivational effect of rewarding stimuli.

(3) Adult rats make 50-kHz calls when playing with other rats (Burgdorf et al. 2008) and when tickled by an experimenter ("heterospecific play")(Panksepp and Burgdorf, 2000; Panksepp and Burgdorf, 2003; Rygula et al., 2012; Popik et al., 2014). Both types of play appear rewarding; conspecific play produces a CPP (Thiel et al. 2008), and rats that have been tickled will subsequently approach the experimenter's hand (Panksepp and Burgdorf, 2000). Tickling has

also been shown to bias responses to positive outcomes during ambiguous cue testing (Panksepp and Burgdorf, 2000; Rygula et al., 2012). Taken together, playful social interactions seem to produce a positive affective state that is reflected by 50-kHz call emission.

(4) Adult rats have also been shown to approach loudspeakers emitting pre-recorded 50-kHz calls, but not other sounds, including 22-kHz calls, time- and amplitude-matched white noise or background noise (Wohr and Schwarting 2007; Sadananda et al., 2008; Seffer et al., 2014; Willuhn et al. 2014).

(5) Receipt of other natural rewards, such as food and access to an exercise wheel, increases 50-kHz call emission (Brenes and Schwarting, 2014; Heyse et al. 2015).

(6) Anticipation of rewards is also associated with high rates of 50-kHz calling, as shown in studies of rewarding electrical brain stimulation (Burgdorf et al., 2000), food reward (Buck et al. 2014b; Opiol et al., 2015), and social contact (Brudzynski and Pniak, 2002).

(7) Food-deprived rats emit fewer 50-kHz vocalizations, presumably reflecting a negative affective state (Brenes and Schwarting, 2014). Sated rats maintain 50-kHz calling although they seek and consume little food; this suggests that these call potentially serve to express some aspect of reward rather than immediate physiological demands.

(8) Rats that are selectively bred for high rates of 50-kHz calling exhibit less anxiety-related behaviour, are less aggressive and more playful (Burgdorf et al., 2009; Brudzynski et al. 2011).

(9) Lastly, intracerebral drug injections, which produce behavioural effects suggestive of positive affect, induce 50-kHz production. For example, 50-kHz calls are emitted following intra-VTA

injections of a μ-opioid agonist, at doses previously found to be rewarding (Burgdorf et al., 2007). Similarly, neuronal activation of the NAcc occurs in response to playback of 50-kHz calls (Sadananda et al., 2008).

The above findings (see summary in Table 1) all suggest that the 50-kHz call rate may serve as a sensitive, direct and non-invasive measure that quantifies the extent to which positive affect is elicited by natural (e.g. food) and drug rewards (e.g. psychostimulants) as well as positive internal states (e.g. social interactions).

Inconsistencies in the evidence for a role of 50-kHz USVs in affect and reward

Not all lines of evidence, however, support a straightforward association between 50-kHz calls and positive affect. For example, 50-kHz vocalizations are emitted during aggressive encounters, (Thomas et al., 1983; Haney and Miczek 1993; Miczek et al. 1995), morphine withdrawal (Vivian and Miczek, 1991), and in response to painful stimuli (Dinh et al. 1999; Niel and Weary 2006). Moreover, not all rewarding drugs produce 50-kHz calls; in a recent study from our laboratory, doses of morphine that produced a clear CPP did not elicit 50-kHz calls (Wright et al. 2012a). In addition to morphine, MDMA, nicotine and alcohol all also produce reward in various behavioural measures (Green and Grahame 2008; De and Dani 2011; Roger-Sanchez et al. 2013) but did not induce 50-kHz USVs (Simola et al., 2012; Simola et al., 2014).

Thus, the simple notion that 50-kHz call rates serve to measure positive affect is most likely incorrect. An alternative hypothesis is that positive affect is represented, not by the rate of vocalization, but by the emission of one or more 50-kHz call *subtypes*.

Table 1 Summary of evidence and inconsistencies for a role of 50-kHz calls as a measure of affect.

Evidence supporting a role for 50-kHz calls in affect		
Reference(s)	**Manipulation and/or stimulus**	**Main result(s)**
Ahrens et al. 2009; Wright et al. 2010; Ma et al. 2010; Wright et al. 2012b; Simola et al. 2014	Systemic administration of amphetamine or cocaine	Increase in 50-kHz call rate
Ahrens et al. 2013	Amphetamine conditioned place preference (CPP)	Larger CPP in rats that produced more 50-kHz vocalizations
Meyer et al. 2011	Response to cocaine cue	More 50-kHz calls following a cocaine injection in rats that also attribute greater incentive salience to a cocaine-cue
Buck et al. 2014	Alcohol administration (vapor)	Alcohol-dependent rats emit more calls in response to the alcohol-associated environment and in anticipation of alcohol receipt
Burgdorf et al. 2008	Play behaviour with a cage mate	Increase in 50-kHz calls emitted during play
Panksepp and Burgdorf 2000; Panksepp and Burgdorf 2003; Rygula et al. 2012; Popik et al. 2014	Experimenter-delivered tickling (‘heterospecific play’)	Rats emit 50-kHz calls when tickled
Wohr and Schwarting 2007; Sadananda et al. 2008; Seffer et al. 2014; Willunh et al. 2014	Approach behaviour	Rats will approach a speaker emitting 50-kHz calls vs. other tones
Brenes and Schwarting 2012	Receipt of food	Rats emit 50-kHz calls in response to food delivery
Heyse et al. 2015	Access to an exercise wheel	Rats seek the exercise wheel and emit 50-kHz calls when presented with it
Burgdorf et al. 2000	Receipt of rewarding electrical brain stimulation (EBS)	Anticipatory 50-kHz calls to EBS
Buck et al. 2014; Opiol et al. 2015	Receipt of food	Anticipatory 50-kHz to food delivery
Brudzynski and Pniak 2002	Social contact	Anticipatory 50-kHz calls to social contact with a conspecific

(continuation) Evidence supporting a role for 50-kHz in affect		
Reference(s)	**Manipulation and/or stimulus**	**Main result(s)**
Brenes and Schwarting 2014	Food deprivation	Food-deprived rats emit fewer 50-kHz vocalizations
Burgdorf et al. 2009; Brudzynski et al. 2011	Selective breeding for 50-kHz calls	Rats bred to emit high rates of 50-kHz calls exhibit less anxiety-related behaviour, are less aggressive and more playful
Burgdorf et al. 2001; Burgdorf et al. 2007; Fendt et al. 2006	Intra-cerebral injections of rewarding drugs	Increase in 50-kHz call emission
Sadananda et al. 2008	Neuronal activation of brain region involved in reward processing	Nucleus accumbens activation following playback of 50-kHz calls
Evidence against a role for 50-kHz calls in affect		
Thomas et al. 1983; Haney and Miczek 1993; Miczek et al. 1995	Aggressive encounters	Increase in 50-kHz call rate during aggressive encounters with a dominant conspecific
Vivian and Miczek 1991	Morphine withdrawal	Occurrence of 50-kHz calls during withdrawal
Dinh et al. 1999; Niel and Weary 2006	Painful stimuli	Increase in 50-kHz calls in response to footshock
Wright et al. 2012a; Simola et al. 2012, Simola et al. 2014	Administration of rewarding drugs	Several rewarding drugs (morphine, MDMA, nicotine and alcohol) failed to induce 50-kHz call emission

The role of dopamine in reward

In 1957, Carlson (Carlsson et al. 1957) first proposed dopamine as a neurotransmitter and since then, its many roles have been extensively studied. Dopamine plays a role in several important functions including learning and motivation (Wise 2004; Everitt and Robbins 2005), working memory (Grecksch and Matties 1981; Goldman-Rakic 1995) and motor output (Pearlstein 2013). Not surprisingly then, disruptions in DA signaling are associated with numerous disorders including Parkinson's disease (Blesa and Przedborski 2014), schizophrenia (bi-Dargham 2014), addiction (Leyton and Vezina 2014) and mood disorders (Lammel et al. 2014).

Most DA-related research has focused on its role in reward processing (Di Chiara and Bassareo 2007; Berridge and Kringelbach 2008; Bromberg-Martin et al. 2010), and the mesolimbic DA circuit is often referred to as a 'reward pathway'. More specifically, DA plays a multitude of roles in reward-related processes: effort (Salamone et al. 2007; Trifilieff et al. 2013), stimulus-reward learning (Flagel et al. 2011), incentive salience (Smith et al. 2011), approach behaviour and reward seeking (Ranaldi 2014), reward-prediction error (Porter-Stransky et al. 2013; Schultz 2013), reward evaluation (McCutcheon et al. 2012; Howe et al. 2013), motivation (Salamone and Correa 2012; Richard et al. 2013) and hedonia (Faure et al. 2010). In recent years, new techniques have allowed us to explore DA's roles with finer temporal and spatial resolution (Wickham et al. 2013). For example, voltammetric recordings have revealed regional variations of phasic DA release in the NAcc in response to natural and drug rewards (Cameron et al. 2014; Shnitko et al. 2014).

While the role of DA in reward-prediction was proposed almost two decades ago (Schultz et al. 1997), only in the past few years have subtleties in the downstream signaling mechanisms emerged. For example, changes in phasic DA concentrations in the NAcc appear sufficient to encode reward-prediction errors necessary for reinforcement learning (Hart et al. 2014). In addition, phasic DA release signals the temporal and spatial proximity of rewards, as well as their value (Howe et al., 2013). Changes in phasic DA dynamics also reveal distinctions between drugs within a given drug class (Vander Weele et al. 2014). The amplitude of dopamine currents have also been shown to reflect an intricate interplay between the size of a reward and the cost in obtaining it (Gan et al. 2010; Hollon et al. 2014). Another recently-emerged technique, namely

optogenetics, has allowed us to further probe the role of VTA DAergic neurons in reward and reinforcement. While selective activation of VTA DAergic neurons produces CPP and rats will actively self-stimulate this region (Tsai et al. 2009; Witten et al. 2011; Ilango et al. 2014a; Ilango et al. 2014b), inhibition of this same VTA neuronal population induces aversion as shown by place avoidance (Ilango et al., 2014b).

Modes of dopamine release

Dopaminergic neurons can signal in two distinct ways: through tonic and phasic DA release (Grace and Bunney 1984a; Grace and Bunney 1984b). Tonic DA release results from regular, pacemaker-like activity of DAergic neurons (Grace and Bunney, 1984b) and produces sustained extracellular DA concentrations (Keefe et al. 1993) that appear important for motivation (Berridge and Robinson 1998; Salamone and Correa 2002) and effort-based decision making (Niv 2007; Kurniawan et al. 2011). Phasic DA release occurs when DAergic neurons burst fire; the latter occurs on a subsecond to second timescale and leads to high concentrations of extracellular DA (Paladini and Roeper 2014). Reuptake of these large quantities of DA occurs rapidly via DA transporters (Chergui et al. 1994; Suaud-Chagny et al. 1995) and therefore released DA remains very close to the synapse (Venton et al. 2003; Floresco et al. 2003). These DA transients occur in response to motivationally relevant events (Brown et al. 2011; McCutcheon et al., 2012; Park et al. 2012), and appear to signal the probability of reward availability (Fiorillo et al. 2003) and also the scalar difference between expected and actual rewards (Schultz 1997). In this way, transient DA release helps the animal to learn to associate relevant environmental cues with important events (Wassum et al. 2013). Phasic DA release is

therefore important for positive reinforcement and learning (Wanat et al. 2009; Willuhn et al. 2010; Wickham et al., 2013).

Furthermore, these two methods of DA release are thought to activate distinct signal transduction cascades through the activation of different postsynaptic G protein-coupled receptors (Floresco et al., 2003; Goto and Grace 2005). More specifically, phasic DA primarily activates low-affinity D1 receptors to facilitate limbic inputs, whereas tonic DA has been shown to increase high-affinity D2 receptor activation (Gonon 1988; Richfield et al. 1989). These distinct signaling events have also been found to produce behaviourally selective effects (Schultz 2007; Tsai et al., 2009; Marinelli and McCutcheon 2014).

The role of DA in 50-kHz USV production

Several neurotransmitters have been shown to play a role in the production of 50-kHz calls (Fu and Brudzynski 1994; Brudzynski and Barnabi 1996; Wintink and Brudzynski, 2001; Arnold et al. 2010; Sadananda et al. 2012; Wright et al., 2012b; Wright et al., 2013; Chapter 2: Scardochio and Clarke, 2013; Wohr et al. 2015). As discussed above, both USVs and DA have been associated with reward and reinforcement and several studies support a role for DA in the production of 50-kHz calls. The main evidence is as follows:

1) 50-kHz calls are reliably increased by systemic or intracerebral administration of psychostimulants that increase DA release and/or prevent DA reuptake (Burgdorf et al., 2001; Wintink and Brudzynski, 2001; Thompson et al., 2006; Ahrens et al., 2009; Ma et al., 2010; Taracha et al. 2012; Wright et al., 2013; Mahler et al. 2013; Simola et al. 2013; Wohr et al., 2015).

2) Electrolytic and dopamine-depleting lesions of mesolimbic structures decrease 50-kHz calls (Burgdorf et al., 2007).

3) Systemic administration of DAergic antagonists decreases both spontaneous and AMPH-induced 50-kHz calls (Wright et al., 2013; Chapter 2: Scardochio and Clarke, 2013). Furthermore, intra-VTA injections of dopaminergic antagonists decrease 50-kHz calling (Burgdorf et al., 2007). Conversely, microinjections of DAergic receptor agonists into the NAcc increased 50-kHz calls (Brudzynski et al. 2012).

4) Electrical stimulation at several levels of the mesolimbic DA system, including the VTA and NAcc, either increases the 50-kHz call rate (Burgdorf et al., 2007) or produces anticipatory 50-kHz calling (Burgdorf et al., 2000).

5) Several behaviours that are accompanied by high rates of 50-kHz calling are associated with elevated extrasynaptic DA concentrations, as follows. Increased tonic DA levels in the NAcc have been reported during heterospecific play (i.e. "tickling") (Hori et al. 2013). Second, increased phasic DA release was observed in the NAcc when rats approached a speaker emitting 50-kHz USVs (Willuhn et al., 2014) or when they viewed a cage mate receiving a reward (Kashtelyan et al. 2014).

In addition to this supporting evidence for a role of DA in the production of 50-kHz calls, there exist several inconsistencies in the literature. First, Williams and Undieh found no effect of systemically administered DA agonists or antagonists on 50-kHz call rate (Williams and Undieh 2010). Second, intra-NAcc microinjection of the D2 *antagonist* haloperidol increased 50-kHz call rate (Thompson et al., 2006). Lastly, increasing DAergic transmission through DA transporter inhibition did not increase the 50-kHz call rate (Wright et al. 2013).

Overview of the methods used in this thesis work

Recording rat USVs

Specialized hardware is required to identify rat vocalizations above the human hearing range. Less than a decade ago, rat USVs were typically recorded using super-heterodyne or frequency-division bat detectors; however, both techniques come with important drawbacks. First, super-heterodyne bat detectors must be tuned to a specific frequency range (typically ± 5 kHz centred on the set frequency), and since 50-kHz vocalizations collectively encompass a much broader range (30-90 kHz), the use of this hardware results in a substantial loss of 50-kHz calls (Sales and Pye 1974; Parsons 2000). Second, with frequency-division bat detectors, the frequency of the emitted USV is divided by a predetermined factor (e.g. 10), making it audible to humans. This division results in distortion of the calls and loss of acoustic features (e.g. harmonics, frequency modulation, etc.) that are essential for call categorization (Parsons, 2000). In the experiments described in this thesis, we have instead used broadband recording methods which capture the acoustic information of rat USVs with minimal information loss. Thus, the microphone signal is amplified and sampled at a high rate (e.g. 250 kHz). The digitized information is then subjected to a Fast Fourier transform in order to resolve different frequency components that can then be visualized in a frequency-time spectrogram (Fig. 1). This method allows us to capture calls that might occur within the relevant frequency range (20-100 kHz) and to extract the acoustic features that are important for call subtyping.

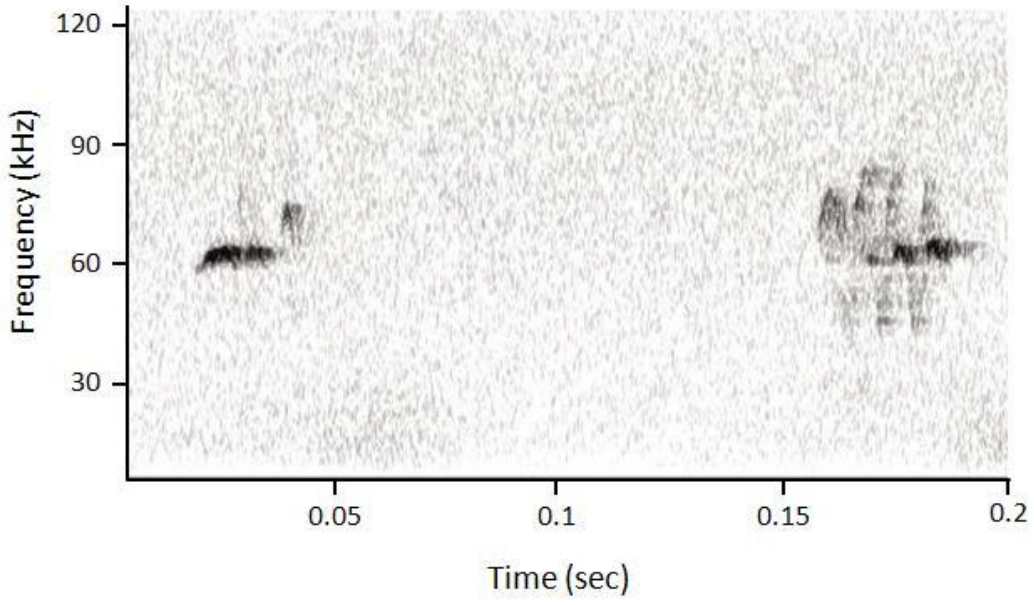

Fig. 1 Example spectrogram containing two amphetamine-induced 50-kHz vocalizations emitted by a single rat, obtained using a high-frequency recording system.

In this thesis, calls were manually selected and subtyped by an experimenter who was blind to the treatment conditions; this can prove to be a very time consuming procedure. It has been a challenge in the field to develop a software program capable of automatically detecting calls with high fidelity, given the complexity of 50-kHz calls (with 14 subtypes so far described) and the acoustic heterogeneity existing within each subtype (Wright et al., 2010). Recently, one group created a program called WAAVES (WAV-file Automated Analysis of Vocalizations Environment Specific) to automatically detect and categorize calls into one of two categories: flat or frequency-modulated (Reno et al. 2013). Since then, another group has published an open-source detection program using template detection created from a large library of calls from three different laboratories (Barker et al. 2014a). Although in its early stages of use, this open-source

detection method is reported to provide up to 90-95% accuracy (dependent on type of call). One improvement for this program would be to expand its use to the detection of 22-kHz calls.

Fast-scan cyclic voltammetry (FSCV)

The FSCV technique relies on the oxidation and reduction of electroactive compounds in the extracellular milieu at potentials that are specific to each analyte (with one exception, see below) (Heien et al. 2003). These redox reactions are induced by applying a range of electrical potentials between a recording ("working") electrode and a reference electrode. The potential (i.e. voltage) at the working electrode is systematically varied; each scan (i.e. recording) involves a rapid upward and downward ramp, and lasts 8.5 ms (Clark et al. 2010). Each scan promotes oxidation and then reduction of the chemical of interest (here: DA). These electrochemical events produce a measurable current (i.e. Faradaic current) flowing between the electrodes. The magnitude of the currents at the peak oxidation and reduction potentials is proportional to the local concentration of the analyte molecule. The background current, due mostly to the capacitive current but also oxygen reduction and other processes at the electrode surface (Takmakov et al. 2010), is stable over short periods of time (e.g. length of scan), allowing it to be subtracted from the readout. This electrochemical output is represented as current against applied potential and is referred to as the background-subtracted cyclic voltammogram (CV). Potential sweeps are applied at a small electrode tip (typically measuring 7 μm in diameter) and can be repeated rapidly (e.g. every 100 ms), thus providing excellent spatial and temporal resolution (Clark et al., 2010). Each CV is specific to the compound of interest and thus serves as an electrochemical signature for its identification. One exception to this electrochemical signature specificity is with catecholamines: noradrenaline and DA are virtually indistinguishable due to their similar redox

potentials (Heien et al., 2003). Therefore, other verifications must be made to distinguish the two. For example, in the work described in this thesis, I selected a brain subregion that contains primarily the catecholamine of interest (DA), and I applied pharmacological agents with known effects on catecholamine concentrations.

In order to perform chronic recordings in freely moving animals, I used the *in vivo* FSCV procedure which was recently developed in the lab of Dr. Paul Phillips (Clark et al., 2010). This procedure, which uses a modified working electrode design (Fortin et al. 2015), permits the detection of phasic catecholamine release ten times per second, in freely moving animals.

Optogenetics

In the 1970s, a basic question - how microscopic organisms sense light - initiated a 40-year investigation that, in 2010, led Dr. Karl Deisseroth to introduce the use of optogenetics to probe mammalian brain structures in vivo (Zhang et al. 2010). Optogenetic procedures use light to produce cellular activation or inactivation via light-sensitive channels (opsins). Briefly, the first step is to build a genetic construct using: 1) specific recombination sites and 2) a gene encoding an opsin. This construct is then inserted into a virus which in turn can be injected into a region of interest. After viral infection, this transgene integrates into the host genome resulting in stable expression of the protein. Rats and mice can be genetically engineered to express an enzyme in specific types of cells. This enzyme recombines a pair of target sequences in the genetic construct, allowing the gene of interest to be activated. Light can then be used to activate the opsin, resulting in cell- and region-specific cellular activation (for review see: Williams and Deisseroth 2013).

The optogenetic approach has allowed us to selectively stimulate midbrain DA neurons to fire transiently (Tsai et al., 2009; Britt et al. 2012). We achieved neurochemical specificity by using genetically engineered rats that expressed Cre recombinase, a tyrosine recombinase enzyme, selectively in DAergic neurons (Tsai et al., 2009; Witten et al., 2011). Viral constructs coding for channelrhoposin-2 were injected into the VTA. Blue light was then used to activate the channelrhodopsins, producing an influx of cations and depolarization of DA neurons. Peak photo-excitation and subsequent neuronal activation occurs within approximately 10 ms, and the opsin channel decays back to a closed state within 10-40 ms (Lorenz-Fonfria and Heberle 2014). Thus, this light-driven neuronal activation can produce phasic DA release in terminal regions (Bass et al. 2010; McCutcheon et al. 2014; Melchior et al. 2015).

In summary, then, optogenetics allows researchers to selectively modulate the activity of specific neurons in model organisms, to investigate the structure and function of neural networks.

STATEMENT OF PURPOSE

As reviewed in Chapter 1, adult rat 50-kHz ultrasonic vocalizations are often associated with contexts and events that putatively involve positive affect and reward. Dopamine has been proposed to play a role in the emission of these calls. However, the published findings concerning the role of dopamine are partially conflicting and do not form a cohesive picture. Therefore, the **overall aim** of this thesis was to provide a re-examination of the role of dopamine in the emission of 50-kHz calls.

Amphetamine reliably increases the rate of 50-kHz calling in adult rats, through both noradrenergic and dopaminergic mechanisms. However, increasing noradrenergic and/or dopaminergic transmission via transporter blockade failed to increase calls. Dopamine transmission can occur in two ways (tonic and phasic), with different behavioural consequences. The **first specific aim** (Chapter 2) was to determine whether activation of dopaminergic receptors was sufficient to increase vocalizations. Our **first main hypothesis** was that selective pharmacological activation of dopamine receptor subtypes would increase 50-kHz call emission. Here, we also co-administered D1 and D2-selective dopaminergic agonists in order to test whether co-activation of these receptors is required for 50-kHz call production.

In Chapter 2, we showed that while dopamine transmission was necessary for the production of ultrasonic vocalizations (i.e. dopamine antagonists decreased calling), tonic receptor stimulation was not sufficient (i.e. dopamine agonists did not increase calls). Unexpectedly, both dopamine agonists and antagonists decreased call rate. This result led to our **second specific aim,** which was to investigate the role of phasic dopamine release on 50-kHz call emission in the drug-free

state. Our **second main hypothesis** (Chapter 3) was that phasic dopamine release would coincide with the production of ultrasonic vocalizations. As a first approach, ultrasonic vocalizations were recorded before and after intermittent electrical stimulation of the medial forebrain bundle. Electrically-evoked phasic dopamine release was verified in the nucleus accumbens by fast-scan cyclic voltammetry. Since electrical stimulation of the medial forebrain bundle is not dopamine-selective, we then used an optogenetic approach to *selectively* activate ventral tegmental area dopaminergic neurons; **we hypothesized** that optogenetic stimulation would elicit ultrasonic vocalizations in a time-locked manner.

Our **third and final main aim** (Chapter 4) was to assess a possible role for phasic dopamine release in the emission of *amphetamine-induced* vocalizations. Specifically, **we hypothesized** that amphetamine-induced 50-kHz calling occurs through the drug's ability to facilitate phasic dopamine release. Accordingly, we predicted that a direct dopamine agonist, by tonically occupying postsynaptic receptors, would impede amphetamine-induced ultrasonic vocalization emission. To this end, phasic dopamine release and ultrasonic vocalizations were recorded following systemic administration of one of five doses of amphetamine. The timing and subtype of calls were studied in relationship to the occurrence of transients, the number of transients and the peak dopamine current.

CHAPTER 2: Inhibition of 50-kHz ultrasonic vocalizations by dopamine receptor subtype-selective agonists and antagonists in adult rats

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Abstract

Rationale Adult rats emit ultrasonic calls at around 22 kHz and 50 kHz that are often elicited by aversive and rewarding stimuli, respectively. Dopamine (DA) plays a role in aspects of both reward and aversion.

Objective To investigate the effects of DA receptor subtype-selective agonists on 22-kHz and 50-kHz call rate.

Methods Ultrasonic calls were recorded in adult male rats that were initially screened with amphetamine to eliminate low 50-kHz callers. The remaining subjects were tested after acute intraperitoneal or subcutaneous injection of the following DA receptor-selective agonists and antagonists: A68930 (D1-like agonist), quinpirole (D2-like agonist), PD 128907 (D3 agonist), PD 168077 (D4 agonist), SCH 39166 (D1-like antagonist), L-741,626 (D2 antagonist), NGB 2904 (D3 antagonist) and L-745,870 (D4 antagonist). The indirect DA/noradrenaline agonist amphetamine served as a positive control.

Results As expected, amphetamine strongly increased 50-kHz call rate. In contrast, D1-, D2- and D3-selective DA receptor agonists when given alone inhibited calling; combinations of D1- and D2-like agonists also decreased call rate. Given alone, the D1-like and D3 antagonists significantly decreased call rate, with a similar trend for the D2 antagonist. Agonist-antagonist combinations also decreased calling. The D4 agonist and antagonist did not significantly affect 50-kHz call rate. 22-kHz calls occurred infrequently under all drug conditions.

Conclusion Following systemic drug administration, tonic pharmacological activation of D1-like or D2-like DA receptors, either alone or in combination, does not appear sufficient to induce 50-kHz calls. Dopaminergic transmission through D1, D2 and D3 receptors appears necessary for spontaneous calling.

Introduction

Adult rat ultrasonic vocalizations (USVs) are commonly divided into two main categories: calls in the 20-30 kHz range, termed 22-kHz USVs, and calls in the 35-90 kHz range, termed 50-kHz USVs (Portfors 2007). These two categories have been proposed to indicate negative and positive affective states, respectively (Knutson et al. 2002; Wöhr and Schwarting 2012). For example, 22-kHz calls are emitted during confrontation with an aggressive conspecific or feline predator, and in response to painful stimuli (Sales 1972b; Cuomo et al. 1988; van der Poel et al. 1989; Blanchard et al. 1991). In contrast, 50-kHz calls have been reported during rough-and-tumble play, copulation, and in anticipation of food delivery (Sales 1972a; Knutson et al. 1998; Burgdorf et al. 2000). However, the association between USV categories and affective valence appears more complex; notably, male rats emit 22-kHz calls after ejaculation (Barfield and Geyer 1972) and emit 50-kHz calls as well as 22-kHz calls during inter-male aggression (Sales 1972b; Thomas et al. 1983).

Evidence from several studies suggests that dopaminergic (DAergic) neurotransmission plays a key role in the emission of 50-kHz calls in adult rats. For example, psychostimulant drugs (amphetamine, methylphenidate and cocaine) which increase dopamine (DA) release and/or block reuptake at the somatodendritic and terminal level (Kalivas et al. 1989; Sulzer 2011) increase the emission of 50-kHz calls (Burgdorf et al. 2001; Wintink and Brudzynski 2001; Thompson et al. 2006; Ahrens et al. 2009; Wright et al. 2010; Meyer et al. 2011; Browning et al. 2011; Brudzynski et al. 2012; Simola et al. 2012; Wright et al. 2012a; Wright et al. 2012b; Wright et al. 2012c). However, all the above indirect DAergic agonists exert additional, non-DAergic effects, and it is therefore important to note that several DAergic antagonists have been

found to markedly inhibit amphetamine-induced 50-kHz calling (Wright et al. 2012b).

Attempts to selectively activate DAergic receptors have, in contrast, produced conflicting findings. For example, 50-kHz call rate was increased by intra-accumbens microinjection of the D2/D3 agonist (quinpirole; Brudzynski et al. 2012) and by a D2-like receptor *antagonist* (haloperidol; Thompson et al. 2006). To date, only two studies have investigated the effects of *systemically*-administered D1- and D2-like drugs on spontaneously-emitted 50-kHz USVs. In the first of these, neither the agonists nor antagonists affected call rate but baseline call rates were low (Williams and Undieh 2010). In the second, both D1-like and D2-like antagonists inhibited calling (Wright et al. 2012b). However, in the two latter studies, the test drugs were only selective for D1-like *vs*. D2-like receptor families rather than individual DA receptor subtypes (Andersen and Jansen 1990; Gehlert et al. 1992; Ruskin et al. 1998; Boulougouris et al. 2009). The main aim of the present study was therefore to investigate the acute effects of DA receptor subtype-selective drugs on 50-kHz calls. To this end, we recorded USVs following acute systemic administration of DA receptor subtype-selective agonists, antagonists and several agonist-antagonist combinations. Given that DA also plays a role in aversion (Bromberg-Martin et al. 2010, Lammel et al. 2012), we simultaneously recorded 22-kHz vocalizations. Finally, since functional synergy is sometimes observed between D1-like and D2-like DA receptors (Clark and White 1987; LaHoste et al. 2000) we also tested combinations of a D1- and a D2-like agonist.

Methods

Subjects

Twenty experimentally-naïve male Long-Evans rats (Charles River Laboratories, St. Constant, Quebec, Canada) were used in each experiment (total of 140 rats). Rats initially weighed 268-356 g at the beginning of the experiment. Subjects were housed two per cage in a temperature- and humidity-controlled colony room (20-22°C, 50-60%). Home cage bedding consisted of laboratory grade Sani-Chips (Harlan Laboratories, Indianapolis, IN). Rats were maintained on a reverse 12:12 h light/dark cycle, with lights off at 0700 h. Behavioural testing took place during the dark phase of the subjects' cycle, between 0800 h and 1300 h. Food and water were available *ad libitum*, except during testing. Subjects were each handled once daily for three minutes, on two days before the first experimental day. Exceptionally, in Experiment 1, subjects were handled on five days before the start of testing. All procedures were approved by the McGill Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care.

Experimental protocol

Initial amphetamine screen Each experiment began with an initial amphetamine screen (Wright et al. 2012c). This served two purposes: (1) to exclude the significant minority of rats that emit few 50-kHz vocalizations in response to systemic amphetamine (Wright et al. 2010), and (2) to increase the acute response to this drug, which was also used as a positive control in later testing. Briefly, rats (n=20) were given an acute injection of 1 mg/kg amphetamine immediately before placement in testing chambers (once daily 20-min session, on three days, spaced two days apart). Ultrasonic vocalizations occurring in the 12^{th}, 14^{th} and 16^{th} minutes of day 3 were counted. The 8

rats with the lowest call numbers were not tested further, leaving a group size of 12.

Drug tests A separate group of rats was used for each experiment (n=12) except Experiments 4 and 8 which were completed with the same group of rats (n=12). Each rat received 5-16 test sessions, depending on the experiment, spaced two days apart. Fully parametric within-subject designs were employed (i.e. each rat was tested under each drug condition). Amphetamine (1 mg/kg IP) served as a positive control throughout. The order of drug treatment within an experiment was as nearly counterbalanced as subject numbers allowed. By visual inspection, physical appearance and any unusual behaviours were noted before all injections, between injections (where applicable) and after each injection and test session.

Drugs

All test drugs, doses, injection timings and routes of administration are shown in Table 1. All doses were chosen based on behavioural effectiveness in other assays (Hoffman and Beninger 1988; Al-Naser and Cooper 1994; Bartoszyk 1998; Hsieh et al. 2004; Millan et al. 2004a; Fenu et al. 2005; Melis et al. 2006; Xi and Gardner 2007). The following drugs were used: the D1-like agonist A68930 hydrochloride, D1-like antagonist SCH 39166 hydrobromide, D2/D3 agonist (-)-quinpirole hydrochloride, D2 antagonist L-741,626, D3 agonist (+)-PD 128907 hydrochloride, D3 antagonist NGB 2904, D4 agonist PD 168077 maleate and the D4 antagonist L-745,870 trihydrochloride. Drugs were purchased from Tocris Bioscience (Minneapolis, MN), except for d-amphetamine (Sigma-Aldrich, Poole, UK), A68930 and quinpirole (Sigma Aldrich, Oakville, ON). All drugs were dissolved in 0.9% sterile saline, with the following exceptions: (1) L-741, 626 was dissolved in 22% DMSO/78% deionized water v/v and (2) NGB 2904 was dissolved in a 5% w/v solution of 2-hydroxypropyl-β-cyclodextrin in deionized water. The timing of each

control (vehicle) injection matched that of the respective drug. Drug solutions were pH-matched to the corresponding vehicle solution (pH 5.6-7.0). All doses are expressed as salt. Drugs were administered in a volume of 1 ml/kg except for: (1) A68930 in Experiments 1 and 5 (2) NGB 2904, and (3) L-741,626, which were all administered in a volume of 2 ml/kg, as were their corresponding vehicles. All drugs were administered by the intraperitoneal (IP) or subcutaneous (SC) route (see Table 1).

Acoustic data acquisition and analysis of ultrasonic vocalizations

The apparatus, testing procedure and acoustic analysis were as previously described (Wright et al. 2012c). Testing was carried out in clear Plexiglas™ experimental boxes (ENV-007CT, Med Associates, St. Albans, VT), each enclosed in a separate melamine compartment that was lined with sound-attenuating acoustic foam (Primacoustic, Port Coquitlam, BC). Condenser ultrasound microphones (CM16/CMPA, Avisoft Bioacoustics, Berlin, Germany) were placed above a small (5 cm diameter) hole, located at the top center of each experimental box. The microphones were located 15-30 cm from the rat. Microphone signals were delivered to an UltraSoundGate 416H data acquisition device (Avisoft Bioacoustics) with a sampling rate of 250 kHz and 16-bit resolution. Avisoft SASLab Pro software (version 5.1.14, Avisoft Bioacoustics, Berlin, Germany) was used for acoustical analysis. Spectrograms were created with a Fast Fourier Transform (FFT) length of 512 points and an overlap of 75% (FlatTop window, 100% frame size) yielding a frequency resolution of 490 Hz and a time resolution of 0.5 ms. Calls were selected manually from spectrograms by an individual masked to treatment conditions.

Data analysis and statistics

Data were analyzed using commercial software (Systat v11, SPSS, Chicago, IL; GraphPad Software, La Jolla, CA). Calls between 20-30 kHz were rarely observed and were not analyzed statistically. Call rate was defined as the total number of 50-kHz calls per minute. Analyzed time bins (see Table 1) were evenly spaced across the session, and the session duration was chosen based on the behavioural time course of each drug. Use of parametric *vs.* non-parametric tests depended on the distribution of the data. For example, non-parametric tests were used where the variances were heterogeneous. Multiple comparison tests were performed using Wilcoxon signed-rank tests. Single comparisons were done using paired t-tests for vehicle conditions in all experiments except Experiment 7. Differences between multiple vehicles were assessed by Friedman's non-parametric analysis of variance. For all tests, a two-tailed *p*-value less than 5% was considered significant.

Results

Initial amphetamine screen, and subsequent saline and amphetamine tests

Since 22-kHz calls were seldom observed, they are reported only under Other Observations (below). During the initial amphetamine screen, the median 50-kHz call rate was 32 calls/min with an interquartile range (IQR) of 5.5-61 (i.e. pooling all 140 rats from all experiments); with the low-callers removed from each experiment (see Methods), the median 50-kHz call rate was 54 calls/min, IQR 40-77 (i.e. pooling the 84 remaining rats). During drug testing blocks, the call rate was much lower under control conditions (i.e. after saline injection, median=3 calls/min, IQR 1-13, n=84 rats) than after amphetamine administration (median=61 calls/min, IQR 28-85). This call-promoting effect of amphetamine was significant in all eight experiments (Wilcoxon's signed-rank test, $Z=2.824$ to 3.059, $p<0.01$ for each).

Experiments 1-4: Receptor subtype-selective dopamine agonist dose-response relationships

For each experiment, the two control (vehicle) tests did not significantly differ with respect to 50-kHz call rate and these data were averaged for each rat.

D1-like agonist A68930 (0.0625-4 mg/kg) significantly decreased the 50-kHz call rate at the three highest doses (Wilcoxon, $Z=2.357$-3.059, $p<0.05$ to $p<0.01$; Figs. 1A and B).

D2/D3 agonist Quinpirole (0.033-1 mg/kg) inhibited 50-kHz calling at all doses tested (Wilcoxon: $Z=1.961$-3.059, $p<0.05$ to $p<0.01$; Figs. 1C and D).

D3 agonist PD 128907 (0.001-1 mg/kg) significantly reduced the 50-kHz call rate at all doses except the second lowest (Wilcoxon, $Z=1.961$-2.825, $p<0.05$ to $p<0.01$; Figs. 1E and F).

D4 agonist PD 168077 (0.033-1 mg/kg) did not significantly affect 50-kHz call rate, except at the second lowest dose (Wilcoxon: $Z=2.118$, $p<0.05$; Figs. 1G and H).

Experiment 5: Combination of D1-like and D2/D3-selective agonists

We next tested the D1-like agonist, A68930, and the D2/D3 agonist, quinpirole, in combination. Low- and high-dose combinations were chosen based on the results of Experiments 1 and 2, i.e. A68930 0.0625 mg/kg + quinpirole 0.033 mg/kg, and A68990 0.25 mg/kg + quinpirole 0.1 mg/kg (Fig. 2). The high-dose combination significantly decreased 50-kHz call rate (Wilcoxon, $Z=2.194$, $p<0.05$), and a similar trend was observed with the low-dose combination (Wilcoxon, $Z=1.836$, $p=0.066$).

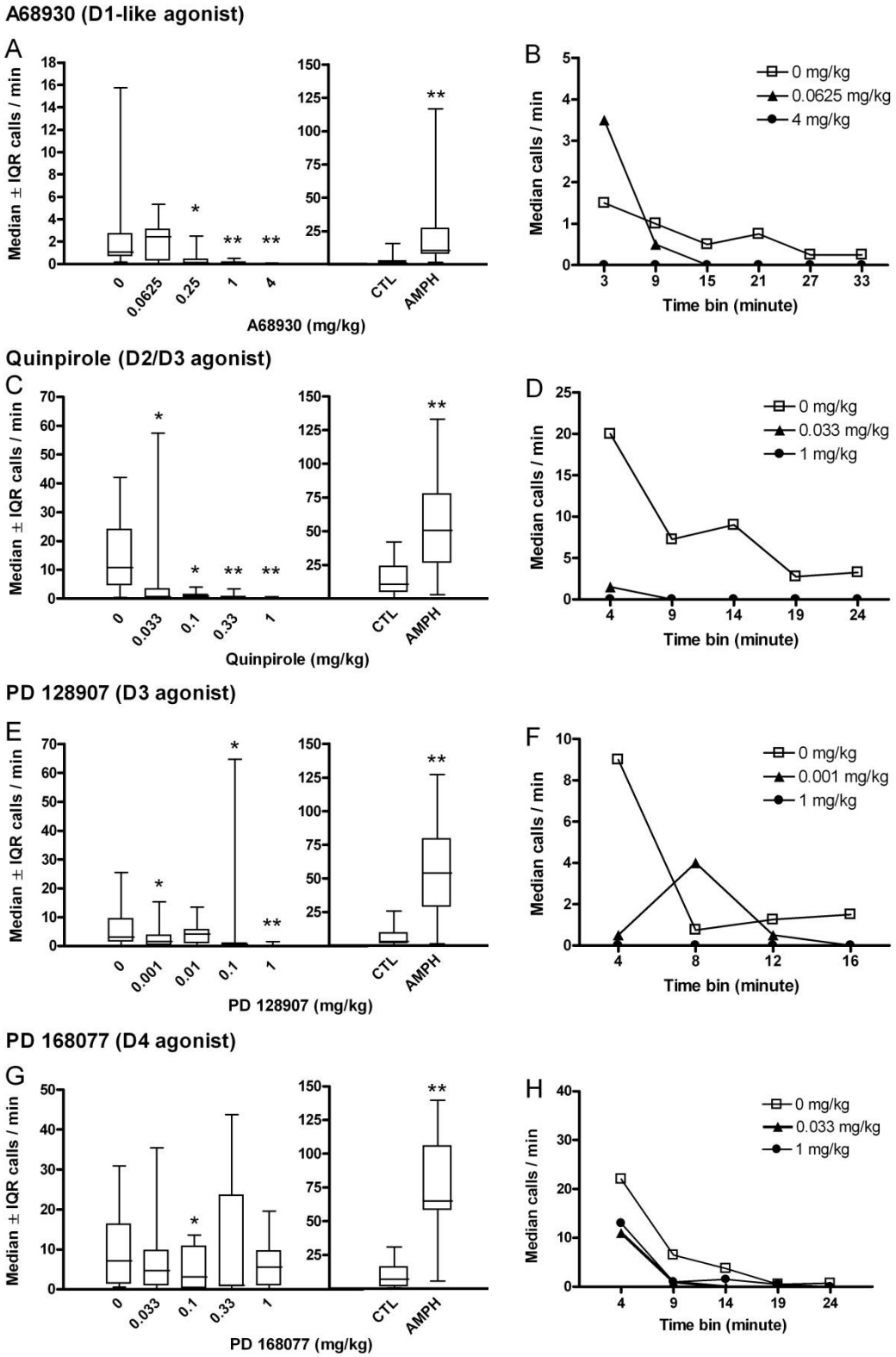
A68930 (D1-like agonist)
A
Median ± IQR calls / min
A68930 (mg/kg)
0
0.0625
0.25
1
4
CTL
AMPH
B
Median calls / min
0 mg/kg
0.0625 mg/kg
4 mg/kg
Time bin (minute)
Quinpirole (D2/D3 agonist)
C
Quinpirole (mg/kg)
0.033
0.1
0.33
D
0 mg/kg
0.033 mg/kg
1 mg/kg
PD 128907 (D3 agonist)
E
PD 128907 (mg/kg)
0.001
0.01
F
0 mg/kg
0.001 mg/kg
1 mg/kg
PD 168077 (D4 agonist)
G
PD 168077 (mg/kg)
H
0 mg/kg
0.033 mg/kg
1 mg/kg

Fig. 1 Experiments 1, 2, 3 and 4: Dose-dependent (**A**, **C**, **E** and **G**) and time-dependent (**B**, **D**, **F** and **H**) effects of DAergic agonists on 50-kHz call rate (n=12 in each panel). Panels **A**, **C**, **E** and **G** are box plots showing median ± IQR (interquartile range). The lowest and highest doses in these left-hand panels are represented as median calls per 1-min time bin in panels **B**, **D**, **F** and **H**, respectively (for the same panels with IQR bars added, see Supplementary **Fig. S1**). Amphetamine (AMPH, 1 mg/kg IP) served as a positive control. *p<0.05, **p<0.01 *vs*. zero dose (Wilcoxon's tests). The same vehicle condition is shown twice in panels **A**, **C**, **E** and **G** (i.e. 0 and CTL).

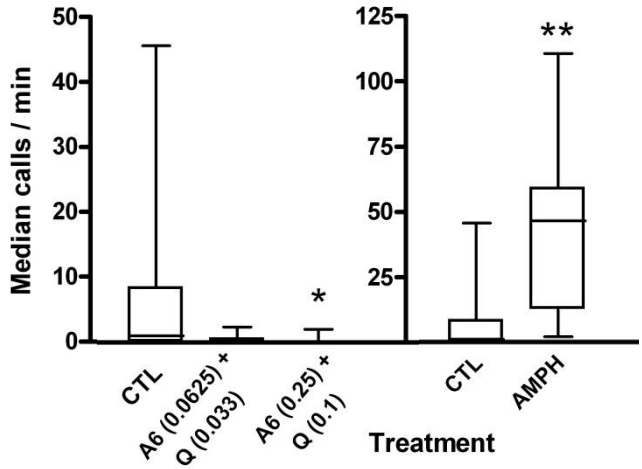

Fig. 2 Experiment 5: Effect of D1-like (A68930; A6) and D2/D3 dopamine agonist (quinpirole; Q) combinations on 50-kHz call rate. Each rat (n=12) was tested under each treatment condition, doses are expressed as mg/kg and were given SC (A6) or IP (Q). Amphetamine (AMPH, 1 mg/kg IP) and vehicle (CTL) served as controls. *p<0.05, **p<0.01 *vs*. CTL (Wilcoxon's tests).

Experiment 6: D3 and D2/D3 agonists in combination with a D3 antagonist

The observed effects of quinpirole (D2/D3 agonist) on call rate in Experiment 2 resembled the call-suppressive effect of the selective D3 agonist PD 128907 from Experiment 3. To test whether quinpirole's effects were due to its actions at the D3 receptor, we administered this drug in combination with a selective D3 receptor antagonist, NGB 2904 (Fig. 3). The call rates in the two control conditions, saline and β-cyclodextrin, did not differ significantly (paired t-test, NS) and were averaged for each rat. Quinpirole and PD 128907, given alone, both significantly decreased calling (Wilcoxon, $Z=2.118$ and 2.001, $p<0.05$). The D3 antagonist NGB 2904 itself did not significantly affect call rate (Wilcoxon, $Z=0.549$, NS) and did not appear to reduce the agonist-induced call suppression (Fig. 3). In the presence of the D3 antagonist, both quinpirole and PD 128907 exerted a residual depressant effect on call rate (antagonist alone vs. antagonist/agonist combination, Wilcoxon, $Z=2.511$ and 1.961, $p<0.05$).

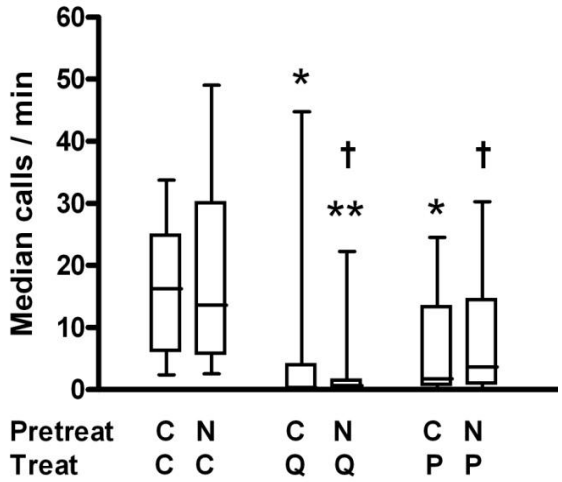

Fig. 3 Experiment 6: The D2/D3 agonist quinpirole (Q, 0.1 mg/kg IP), and the D3 agonist PD 128907 (P, 0.1 mg/kg SC) administered with either vehicle pretreatment (i.e. C, average of both vehicles used) or the D3 antagonist NGB 2904 (N, 0.1 mg/kg IP). Amphetamine (AMPH, 1 mg/kg IP) served as a positive control. Each rat (n=12) was tested under all conditions. *$p<0.05$,

**p<0.01 *vs*. control (C). The same control condition is shown in both panels. †p<0.05 *vs*. antagonist alone (Wilcoxon's tests).

Experiment 7: D1-like, D2/D3 and D3 selective agonists in combination with selective antagonists

In Experiment 6, the D3 antagonist NGB 2904 failed to counter the call-suppressant effect of the D2/D3 agonist quinpirole and the D3 agonist PD 128907. Therefore, we next tested these agonists in combination with a higher dose of NGB 2904 (i.e. 2 mg/kg instead of 1 mg/kg). The same two agonists were also tested together with the D2-selective antagonist L-741,626. Within the same drug testing block, the D1-like agonist (A68930) was tested in combination with a D1-like antagonist (SCH 39166).

The call rates in the three control conditions (saline, DMSO and β-cyclodextrin) were not significantly different and were averaged. As shown in Fig. 4, the D1-like antagonist SCH 39166 decreased call rate when given alone (Wilcoxon, Z=2.903, p<0.01); this drug also produced lethargy within a few minutes of injection. The D1-like agonist A68930 also tended to inhibit calling (Wilcoxon, Z=1.726, p=0.084), and exerted a marginally significant residual effect in the presence of SCH 39166 (Wilcoxon, Z=1.962, p=0.0498). The combination of D1-like antagonist and agonist virtually abolished 50-kHz calling (Wilcoxon, Z=3.060, p<0.01).

The D3 antagonist NGB 2904 decreased call rate when given alone (Wilcoxon, Z=1.962, p<0.05) and the D2 antagonist L-741,626 also tended to decrease calling (Wilcoxon, Z=1.726, p=0.084). In the absence of antagonist, quinpirole and PD 128907 significantly decreased 50-kHz calls, as found earlier (Wilcoxon, Z=2.903 and 3.061, p<0.01). Following D2-selective antagonist pretreatment, quinpirole exerted residual call-suppressant effects (i.e. when compared

to antagonist alone), while PD 128907 did not (Wilcoxon, Z=2.805, p<0.01; Z=0.297, NS). Conversely, following D3 antagonist treatment, PD 128907 but not quinpirole exerted significant residual call-suppressant effects (Wilcoxon, Z=2.654, p<0.01; Z=1.939, p=0.053, respectively).

Experiment 8: D4 selective antagonist

The D4 antagonist, L-745 870 (1 mg/kg IP) did not significantly affect 50-kHz call rate (Wilcoxon, Z= 1.784, NS). The median call rates under drug and saline were, respectively, 0.7 calls/min (IQR 0.1-3.2) and 1.2 calls/min (IQR 0.2-5).

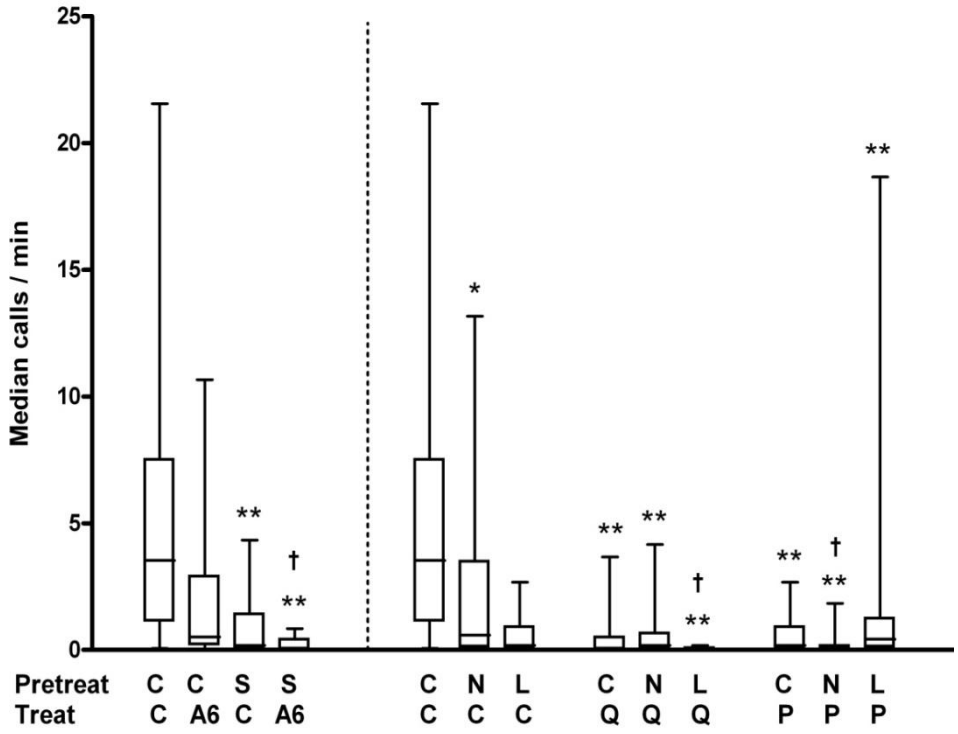

Fig. 4 Experiment 7: Effects of dopamine agonists given alone (i.e. with control (C) pretreatment), or in combination with their corresponding antagonist. All drugs were given IP or SC (see Table 1): D1-like agonist, A68930 (A6); D2/D3 agonist, quinpirole (Q); D3 agonist, PD

128907 (P); D1-like antagonist, SCH 39166 (S); D2 antagonist, L-741,626 (L) and D3 antagonist, NGB 2904 (N). Amphetamine (A, 1 mg/kg IP) served as a positive control. *p<0.05, **p<0.01 *vs*. C (control = mean of the three vehicles used). The same drug-free control condition (i.e. C/C) is represented three times. †p<0.05 *vs*. corresponding antagonist alone (Wilcoxon's tests), n=12.

Other observations

Novel 22-kHz calls intermingled with 50-kHz calls We observed frequency-modulated long 22-kHz calls that are different from the typically reported long 22-kHz calls. More specifically, these calls comprised a long (400-1520 ms) low-frequency (24-29 kHz) component, preceded and/or followed by a high-frequency (41-61 kHz) component. These calls, which were intermingled with 50-kHz calls, occurred infrequently (i.e. a total 8 calls, found in 2 out of 12 rats in Experiment 6), and only under amphetamine. In contrast, constant-frequency 22-kHz calls were not observed in rats receiving amphetamine, and seldom occurred under other drug conditions (14 calls in 2 rats).

Audible calls In Experiment 1, the two highest doses of A68930 (1 and 4 mg/kg), caused audible calls approximately one hour after the end of the session (two hours post-injection), in 3 of the 12 rats tested. These calls were emitted in their home cage in the presence of their cage mate and stopped immediately upon social separation.

Discussion

The present study provides the first report that spontaneous 50-kHz call rates can be reduced by systemic administration of DAergic agonists. Call inhibition occurred not only with D1-like, D2 and D3 receptor-selective agonists, but also with DAergic antagonists and agonist/antagonist combinations. These call rate-suppressive effects contrasted strongly with the well-established rate-enhancing effects of the indirect DA/noradrenaline (NA) agonist amphetamine that occurred reliably in the same animals.

Call-suppressive drug effects vs. motor inhibition

Several classes of DAergic drugs affect motor function (for review see Jackson and Westlind-Danielsson 1994). In the present study, the majority of DAergic agents decreased 50-kHz call rate, but only SCH 39166 (D1-like antagonist) produced visible signs of motor impairment or lethargy. For the remaining drugs, there was no consistent relationship with motor output. First, at the doses used there was no visible sign of catalepsy, which is consistent with literature reports (Millan et al. 1998; Millan et al. 2000; Banasikowski and Beninger 2012). Second, quinpirole inhibited calling not only at low, locomotor depressant doses (0.033 and 0.1 mg/kg; Schaub et al. 1997; Schindler and Carmona 2002), but also at higher doses reported to increase locomotor activity (LA) (1-10 mg/kg; Horvitz et al. 2001). Third, the D3 agonist PD 128907 inhibited calling even at low doses that would not be expected to affect LA (Gyertyan and Saghy 2004; Millan et al. 2004b). Fourth, NGB 2904 (D3 antagonist) decreased the call rate at doses that have been shown to increase spontaneous LA (Pritchard et al. 2007). Fifth, A68930 (D1-like agonist) and L-741,626 (D2 antagonist) are reported not to affect LA (Deveney and Waddington 1997; Clifford and Waddington 2000; Isacson et al. 2004; Nergardh et al. 2005; Koffarnus et al. 2011;

Chang et al. 2011) at doses which inhibited calling. In conclusion, we cannot exclude the possibility that some drugs at certain doses (notably high-dose SCH 39166 and low-dose quinpirole) reduced 50-kHz call rates by inhibiting motor function. However, the present findings also provide examples where USV emission and locomotor activity can be dissociated, as previously reported with other drugs (Burgdorf et al. 2001; Natusch and Schwarting 2010; Wright et al. 2012a).

D1-like, D2/D3- and D3-selective agonists alone decreased 50-kHz vocalizations

In the present study, all DAergic agonists, with the possible exception of the D4-selective agonist, decreased the 50-kHz call rate. Only one previous study has reported the effects of acute, systemically-administered D1- and D2-like selective agonists on spontaneous 50-kHz calling (Williams and Undieh 2010); neither SKF 38393 (D1-like) nor quinpirole (D2/D3) exerted any detectable effect on 50-kHz call rate. Several procedural factors could readily account for differences between the two studies. These factors include the specific drugs used (only quinpirole was common to both), the route of drug administration (i.e. IP *vs.* SC), rat strain, and the recording and analysis methodology. Importantly, in the earlier study call rates under saline were extremely low (e.g. ~5 calls/hour), impeding detection of any inhibitory drug effects.

Combinations of D1-like and D2/D3-selective agonists decreased 50-kHz vocalizations

Concurrent activation of postsynaptic D1-like and D2-like receptors appears to be required for the expression of several DAergic agonist-induced behaviours (Clark and White 1987; Dall'olio et al. 1988; Wachtel et al. 1989; Garrett and Holtzman 1994; Capper-Loup et al. 2002; Hasbi et al. 2011; Ikemoto et al. 1997). To address whether concurrent activation of D1 and D2 receptors

is sufficient to elicit USVs, we administered A68930 and quinpirole in combination. Our lower dose of quinpirole (0.033 mg/kg) would selectively target inhibitory DA autoreceptors (Widzowski and Cory-Slechta 1993), whereas the higher dose (0.33 mg/kg) would be expected to act predominantly at postsynaptic D2 receptors (Cory-Slechta et al. 1996). In the present study, both dose combinations inhibited calling. This result contrasts with a clear stimulant effect reported after systemic administration of the D1/D2-like DAergic agonist apomorphine (Williams and Undieh 2010). However, apomorphine may also have exerted non-DAergic actions at the high dose administered (2 mg/kg SC), for example at adrenergic and 5-HT receptors (Millan et al. 2002; Newman-Tancredi et al. 2002).

The observed effects of systemically administered DAergic agonists suggest that DA receptor activation in multiple brain regions is insufficient to induce calling. Consistent with this conclusion, we recently observed that the call-enhancing effect of systemically administered amphetamine is critically dependent on both dopaminergic and adrenergic receptor mechanisms (Wright et al. 2012b; Wright et al. 2012c).

DA receptor antagonists decreased 50-kHz call rate without affecting agonist-induced inhibition

Dopaminergic antagonists were initially reported to have no effect on spontaneous rates of 50-kHz vocalization after systemic administration (Wintink and Brudzynski 2001; Williams and Undieh 2010), but in both these studies the low basal rates of calling could potentially have masked any inhibitory effects. More recently, we observed a suppression of 50-kHz calling following systemic administration of the D1- and D2-like antagonists SCH 39166 and raclopride (Wright et al. 2012b). Extending the latter observations, the D1-like and D3-selective antagonists tested in the present study both decreased the 50-kHz call rate, with a similar trend for the D2-

selective antagonist. Taken together, the inhibitory effects of systemically-administered DAergic antagonists suggest that DA receptors are necessary for USV emission.

In the present study, the D2- and D3-selective antagonists did not significantly inhibit the effects of their respective agonists. It is likely that the antagonist doses were sufficiently high; first, these drugs appeared to inhibit calling when given alone, and second, comparable doses were effective in other behavioural assays (Fenu et al. 2005; Melis et al. 2006; Collins et al. 2007; Xi and Gardner 2007). In the latter studies, off-target actions appear improbable since these drugs are reported to be highly receptor-selective (McQuade et al. 1991; Kebabian et al. 1992; Levant et al. 1993; Pugsley et al. 1995; Bowery et al. 1996; Glase et al. 1997; Patel et al. 1997; Yuan et al. 1998).

Comparisons with amphetamine and cocaine

The inhibition of 50-kHz calling by D1-like and D2-like agonists is particularly striking when set against the robust call stimulation associated with systemic administration of the indirect agonists amphetamine (Wintink and Brudzynski 2001; Thompson et al. 2006; Wright et al. 2010; Simola et al. 2012) and cocaine (Mu et al. 2009; Williams and Undieh 2010; Meyer et al. 2011; Wright et al. 2012c). Psychostimulant drugs, *via* presynaptic actions, enhance NA as well as DAergic transmission (Kuczenski et al. 1995; Kuczenski et al. 1997; Berridge and Stalnaker 2002), and NAergic mechanisms are clearly critical to amphetamine-induced 50-kHz calling (Wright et al. 2012c). However, a NAergic contribution does not readily explain why DA receptor agonists and antagonists both decreased call rate. Another neuropharmacological difference between amphetamine/cocaine and direct DAergic agonists is that, according to recent *in vivo* voltammetric evidence, amphetamine and cocaine both enhance phasic DAergic signaling

to an important degree (Cheer et al. 2007; Aragona et al. 2008; Ramsson et al. 2011a; Ramsson et al. 2011b). Transient DA release events are known to occur spontaneously (Wightman and Robinson 2002; Schultz 2007), and their postsynaptic impact would likely be masked after administration of DA receptor agonists, antagonists, and their combination. Therefore, based on the present USV findings, we propose the hypothesis that 50-kHz vocalizations (or certain call subtypes) are driven by DA transients.

Behavioural significance of decreased 50-kHz call rate

The relationship of 50-kHz calls with conventional measures of drug reward has been little explored. Specifically, the psychostimulants amphetamine and cocaine, after IP or SC administration, reliably produce CPP (0.5-2 mg/kg and 4-20 mg/kg respectively; Tzschentke 1998) and acutely promote 50-kHz USVs (AMPH 0.5-2 mg/kg, cocaine 10-20 mg/kg; see above for references) whereas morphine can produce a CPP without a concomitant increase in unconditioned USV emissions (Wright et al. 2012a). The present study provides further evidence that unconditioned drug effects on 50-kHz call rate do not necessarily match the conditioned drug effects that are revealed in the CPP/CPA procedure. Our test drugs that decreased 50 kHz calling either (1) produced CPP or no effect (quinpirole; Hoffman and Beninger 1988; Graham et al. 2007), (2) produced CPA or no effect (SCH 39166; Acquas and Di Chiara 1994; Spina et al. 2006), or (3) produced CPP or CPA, even at the same dose (PD 128907; Khroyan et al. 1997; Gyertyan and Gal 2003). Lastly, no published CPP/CPA data appear available for A68930, PD 168077, L-741,626, L-745,870 or NGB 2904.

Several groups have proposed that 50-kHz calls may represent a behavioural expression of positive affect (Cuomo and Cagiano 1987; Knutson et al. 2002; Panksepp and Burgdorf 2003; Brudzynski 2007; Mallo et al. 2009; Barker et al. 2010; Browning et al. 2011; Burgdorf et al. 2011; Hamdani and White 2011). In the present study, most DAergic antagonists *and agonists* inhibited calling, and on this basis we speculate that a decrease in 50-kHz call rate may not necessarily reflect a negative shift in affect, but rather a response to an unfamiliar stimulus or context.

Limitations

Route of administration The present study demonstrated that systemically-administered DAergic agonists and antagonists, given alone, decreased calling. Inhibition of calling by DAergic agonists indicates that DA receptor activation in multiple brain regions is insufficient to induce calling, whereas the inhibitory effects produced by DAergic antagonists suggest that DA receptors are necessary for USV emission. These conclusions run counter to findings from two studies based on intra-accumbens infusions of DAergic agents. In one study, the D2/D3 agonist quinpirole increased 50-kHz calling, while neither D2- nor D3-selective antagonists produced a significant effect (Brudzynski et al. 2012). In the other study, the D1- and D2-like antagonists SKF 32957 and raclopride did not alter 50-kHz call rate, whereas the D2-like haloperidol increased it (Thompson et al. 2006). Taken together, these findings highlight the importance of route of administration and raise the possibility of both inhibitory and excitatory DAergic influences on 50-kHz call emission.

Adverse drug effects High doses of A68930 (1.2 and 3.7 mg/kg) have been reported to trigger motor seizures in adult rats (DeNinno et al. 1991). However, no such effect was noted in several

other studies (Salmi 1998; Salmi and Ahlenius 2000; Isacson et al. 2004; Nergardh et al. 2005), including at the two highest doses tested here (1 and 4 mg/kg; D'Aquila et al. 1994; Deveney and Waddington 1997). Although we did not observe seizures in our rats, we cannot exclude the possibility that our rats suffered convulsions while in the testing chamber since they were not video recorded.

Pharmacology Each DA receptor subtype was probed with a single agonist and antagonist. However, these agents were chosen from the most target-selective available. To our knowledge, no agonists or antagonists currently discriminate between D1 and D5 receptors; for example, the D1-like agonist A68930 and antagonist SCH 39166 have near-equal *in vitro* affinities for D1 and D5 (Tice et al. 1994; Nergardh et al. 2005). In addition, at the start of each experiment, rats underwent an initial screen comprising three spaced injections of amphetamine; we cannot exclude the possibility that this amphetamine exposure affected subsequent calling to other DAergic agents.

Call-subtype analysis Call subtype analysis was not feasible in view of the low overall call rates (often <4 calls per minute) following receptor subtype-selective agonist and antagonist administration.

Conclusion

Overall, the literature is mixed with regards to the role of dopamine in ultrasonic vocalizations and whether these vocalizations may represent affective state. Here we have shown that, following systemic drug administration, tonic pharmacological activation of dopamine receptors is not sufficient to increase 50-kHz vocalization call rate, whereas D1, D2 and D3 receptors may all be necessary for spontaneous calling. The observed drug effects require further investigation

with respect to their neurochemical underpinnings (e.g. DA transients) and behavioural significance (e.g. interpretations not based on affect). Elucidation of the neurochemical events underpinning USV emission may provide a clearer understanding of the affective information that these vocalizations putatively convey.

Table 1: Summary of drug conditions for each experiment

Table 1 Summary of drug conditions for each experiment														
Exp	**Pretreatment**						**Treatment**						**Analysis**	
	Drug		Dose (mg/kg)	Route	Time before testing (min)		Drug		Dose (mg/kg)	Route	Time before testing (min)		Session length (min)	Sampling intervals (minute)
1	---	---	---	---	---		D1-like agonist	A68930	†0, 0.0625, 0.25, 1, 4	SC	20		40	4, 10, 16, 22, 28, 34
2	---	---	---	---	---		D2/D3 agonist	quinpirole	0, 0.033, 0.1, 0.33, 1	IP	5		30	5, 10, 15, 20, 25
3	---	---	---	---	---		D3 agonist	PD 128907	0, 0.001, 0.01, 0.1, 1	SC	10		20	5, 9, 13, 17
4	---	---	---	---	---		D4 agonist	PD 168077	0, 0.033, 0.1, 0.33,1	IP	15		30	5, 10, 15, 20, 25
5	D1-like agonist	A68930	†0.0625	SC	20		D2/D3 agonist	quinpirole	0.033	IP	5		30	5, 10, 15, 20, 25
	D1-like agonist	A68930	†0.25	SC	20		D2/D3 agonist	quinpirole	0.1	IP	5		30	5, 10, 15, 20, 25
6	D3 antagonist	NGB 2904	0, 1	IP	30		D2/D3 agonist	quinpirole	0, 0.1	IP	5		20	5, 9, 13, 17
	D3 antagonist	NGB 2904	0, 1	IP	30		D3 agonist	PD 128907	0, 0.1	SC	10		20	5, 9, 13, 17
7	D1-like antagonist	SCH 39166	0, 0.1	SC	30		D1-like agonist	A68930	0, 0.1	SC	20		40	7, 13, 19, 25, 31, 37
	D2 antagonist	L-741,626	†0, 1	SC	30		D2/D3 agonist	quinpirole	0, 0.1	IP	5		40	7, 13, 19, 25, 31, 37
	D2 antagonist	L-741,626	†0, 1	SC	30		D3 agonist	PD 128907	0, 0.1	SC	10		40	7, 13, 19, 25, 31, 37
	D3 antagonist	NGB 2904	†0, 2	IP	30		D2/D3 agonist	quinpirole	0, 0.1	IP	5		40	7, 13, 19, 25, 31, 37
	D3 antagonist	NGB 2904	†0, 2	IP	30		D3 agonist	PD 128907	0, 0.1	SC	10		40	7, 13, 19, 25, 31, 37
8	D4 antagonist	L-745,870	0, 1	IP	30		---	---	---	---	---		40	7, 13, 19, 25,31, 37

†Given in a volume of 2 ml/kg (otherwise, given in a volume of 1 ml/kg)
In all experiments, each rat was also tested with amphetamine (1 mg/kg IP, positive control) within the counterbalanced design

Connecting Text 1

The previous chapter suggested that DA transmission is necessary but not sufficient for the emission of spontaneously-emitted 50-kHz ultrasonic vocalizations (USVs); while selective DA antagonists decreased calling, so did the selective agonists. To try to understand this common effect, we explored the different dopaminergic (DAergic) signaling mechanisms, i.e. tonic vs. phasic DA release. DA antagonists, acting at postsynaptic DAergic receptors, would inhibit both types of transmission. DA agonists would produce tonic activation of these receptors, but in so doing would blunt the impact of phasic DA release events. Thus, a common effect of DA antagonists and agonists is to mitigate phasic DA events. We therefore hypothesized that it is these *phasic* DA events that are involved in the emission of 50-kHz calls.

CHAPTER 3: Phasic dopamine release in relation to rat ultrasonic vocalizations: insights from electrical and optogenetic brain stimulation

Tina Scardochio, Ivan Trujillo-Pisanty, Peter Shizgal, Paul B.S. Clarke

Abstract

Rationale Adult rats emit ultrasonic vocalizations (USVs) at around 50-kHz that commonly occur in contexts that putatively engender positive affect. While several reports indicate that dopaminergic (DAergic) transmission plays a role in the emission of 50-kHz calls, the pharmacological evidence is mixed. Different modes of dopamine (DA) release (i.e. tonic and phasic) could potentially explain this discrepancy.

Objective To investigate the potential role of *phasic* DA release on 50-kHz call rate.

Methods In Experiment 1, USVs were recorded in adult male rats following unexpected electrical stimulation of the medial forebrain bundle (MFB). In parallel, phasic DA release in the nucleus accumbens (NAcc) was recorded using fast-scan cyclic voltammetry. In Experiment 2, USVs were recorded following response-contingent or non-contingent optogenetic stimulation of ventral tegmental area (VTA) DAergic neurons. Four 20-s schedules of optogenetic stimulation were used: fixed-interval, fixed-time, variable-interval and variable-time.

Results Brief electrical stimulation of the MFB increased both 50-kHz call rate and phasic DA release in the NAcc. During optogenetic stimulation sessions, rats initially called at a high rate comparable to that observed following reinforcers such as psychostimulants. Although optogenetic stimulation maintained reinforced responding throughout the 2-hour session, the call rate declined to near zero within the first 30 minutes. The trill call subtype predominated following both electrical and optical stimulation.

Conclusion

The occurrence of electrically-evoked 50-kHz calls, time-locked to phasic DA (Experiment 1), provides correlational evidence supporting a role for phasic DA in USV production. However, in Experiment 2, the temporal dissociation between calling and optogenetic stimulation of VTA DAergic neurons suggests that phasic mesolimbic DA release is not sufficient to produce 50-kHz calls. The emission of the trill subtype of 50-kHz calls potentially provides a marker distinguishing positive affect from positive reinforcement.

Introduction

Adult rats produce two categories of ultrasonic vocalizations (USVs) (Portfors 2007; Clarke and Wright 2015): 22-kHz (range: 20-30 kHz) and 50-kHz (30-90 kHz). Calls in both categories are proposed to play a role in communication (for review see: Seffer et al. 2014). In addition to their proposed communicative role, the 22- and 50-kHz call categories appear to reflect negative and positive affective states, respectively (Knutson et al. 2002). Thus, while 22-kHz calls are commonly associated with aversive situations (Litvin et al. 2007, Mahler et al. 2013), 50-kHz calls have been detected during a variety of rewarding events, such as rough-and-tumble play and administration of psychostimulant drugs (Burgdorf et al. 2001; Panksepp and Burgdorf 2003; Wright et al. 2010).

Several neurotransmitters appear to play a role in the emission of 50-kHz USVs in adult rats (Fu and Brudzynski 1994; Panksepp and Burgdorf 2000; Wintink and Brudzynski 2001; Fendt et al. 2006; Burgdorf et al. 2007; Arnold et al. 2010; Wright et al. 2012; Sadananda et al. 2012; Wright et al. 2013; Manduca et al. 2014; Wohr et al. 2015). Here, dopamine (DA) has received particular attention, given its well-established role in motivation and reward (Bromberg-Martin et al. 2010; Ikemoto 2010; Covey et al. 2014; Ranaldi 2014). A variety of dopaminergic (DAergic) manipulations alter the rate at which adult rats emit 50-kHz calls (Burgdorf et al. 2000; Burgdorf et al., 2001; Burgdorf et al., 2007; Williams and Undieh 2010; Brudzynski et al. 2012; Simola et al. 2013); in particular, DAergic transmission in the nucleus accumbens (NAcc) appears both necessary and sufficient for call emission, as evidenced by studies using DA-targeted lesions and intracerebral microinjection of DAergic drugs (Burgdorf et al., 2001; Burgdorf et al., 2007). Accordingly, we and others have found that both amphetamine (AMPH)-

induced and spontaneous 50-kHz vocalizations are profoundly inhibited by systemically-administered D1 and D2 antagonists (Wright et al., 2013; Chapter 2: Scardochio and Clarke 2013; Wohr et al., 2015). However, some DAergic drugs have produced unexpected effects after systemic administration: (1) direct DAergic agonists *inhibited* 50-kHz calling across a wide dose range (Chapter 2: Scardochio and Clarke, 2013), and (2) the DA transporter blocker GBR 12909 failed to mimic AMPH's stimulatory effect on 50-kHz call emission, even when combined with a noradrenaline transporter blocker (Wright et al., 2013).

In the present study, we have asked whether these apparently conflicting findings might reflect differential drug effects on two distinct modes of DA transmission: tonic and phasic. In the absence of salient stimuli, midbrain DAergic neurons display a tonic pacemaker-like activity, maintaining a stable and low DA extracellular concentration ('tone') in terminal structures such as the nucleus accumbens (NAcc) (Grace and Bunney 1984). Salient stimuli such as primary rewards induce neuronal burst firing, resulting in phasic DA release associated with a rapid and transient increase in extracellular DA concentrations (Redgrave et al. 2008). Several observations suggest that 50-kHz call emission may be associated with *phasic* DA release. Notably, 50-kHz vocalizations have been evoked by several manipulations that have been shown to increase phasic DA release: experimenter-delivered 'tickling' (Hori et al. 2013), playback of 50-kHz vocalizations (Willuhn et al. 2014) and the presence of a conspecific receiving reward (Kashtelyan et al. 2014). Rats will also emit 50-kHz calls in anticipation of electrical stimulation of the medial forebrain bundle (MFB) that would be expected to increase phasic DA release in terminal areas such as the NAcc (Burgdorf et al., 2000). If phasic DA transmission promotes USV emission, this could also reconcile several pharmacological findings: (1) AMPH and

cocaine reliably induced 50-kHz calling and increased phasic DA release (Cheer et al. 2007; Wright et al., 2010; Willuhn et al. 2012; Wright et al., 2013; Daberkow et al. 2013; Covey et al., 2014), (2) tonic activation of postsynaptic receptors by selective DA receptor agonists inhibited spontaneous calling (Chapter 2: Scardochio and Clarke, 2013), and (3) the DAT blocker GBR 12909, expected to elevate only *tonic* DA transmission, did not increase USV emission (Wright et al., 2013).

The aim of the present study was therefore to test whether phasic DA release events drive 50-kHz calls in adult rats. Previously, Burgdorf et al. (2007) showed that electrical stimulation of the medial forebrain bundle (MFB) evoked 50-kHz call emission and that these calls were decreased by the DA antagonist flupenthixol. However, flupenthixol has non-DAergic effects, notably on 5-HT receptors (Kuhn et al., 2000), and in addition phasic DA release was inferred rather than measured. Therefore, to directly assess the involvement of phasic DA release, our first experiment asked whether electrical stimulation of the MFB would elicit USVs using parameters that evoked phasic DA release events detected using fast-scan cyclic voltammetry. The second experiment investigated whether phasic DA activity was *sufficient* to induce 50-kHz calls. Here, we recorded USVs during optogenetic stimulation that was designed to selectively activate VTA DAergic neurons. Two parameters of reinforcement were investigated: (1) expected *vs.* unexpected stimulation to test for anticipatory calling and (2) contingent *vs.* non-contingent stimulation to allow for comparison with previous studies using electrical stimulation.

Methods

Acquisition and identification of ultrasonic vocalizations

The testing procedure and acoustic analysis were in part, as previously described (Wright et al. 2010 RM692). For the initial amphetamine screen, clear Plexiglas™ experimental boxes (ENV-007CT, Med Associates, St. Albans, VT) were used for testing and each was enclosed in a separate melamine compartment lined with sound attenuating acoustic foam (Primacoustic, Port Coquitlam, BC). For the optogenetic and fast-scan cyclic voltammetry (FSCV) experiments, Clear Plexiglas™ experimental boxes (optogenetic: ENV-007CT, Med Associates, St. Albans, VT, FSCV: made in-house) were also used but sound attenuating acoustic foam was not utilized. Condenser ultrasound microphones (CM16/CMPA, Avisoft Bioacoustics, Berlin, Germany) were located at the top center of each test box, 30-60 cm from the rat. Microphone signals were delivered to an UltraSoundGate 416H data acquisition device (Avisoft Bioacoustics, Glienicke, Germany) with a sampling rate of 250 kHz and 16-bit resolution. Avisoft SASLab Pro software (version 5.1.14, Avisoft Bioacoustics, Berlin, Germany) was used for acoustical analysis. Spectrograms were created with a fast Fourier transform (length 512 points, overlap 75%, FlatTop window, 100 % frame size) yielding a frequency resolution of 490 Hz and a time resolution of 0.5 ms. Calls were selected manually from spectrograms by an individual masked to treatment conditions. Call rate was defined as the total number of 50-kHz calls per 5 minutes, unless otherwise noted. All calls were categorized into one of 14 subtypes, as defined by Wright et al. 2010, plus two additional categories that are rarely observed (~1% of calls): 'unclassifiable'(call was not loud enough, or noise was present, which prevented accurate subtyping) and 'miscellaneous' (call was visible but did not clearly fit one of the 14 subtypes).

Statistics

Data were analyzed using commercial software (Systat v11, SPSS, Chicago, IL; GraphPad Software, La Jolla, CA). Except during the MFB stimulation experiment, USVs between 20- and 30-kHz were rarely observed and were not analyzed statistically; otherwise, all USVs refer to the 50-kHz subtype. Nonparametric tests were used when the data suggested that parametric test assumptions (e.g. variance homogeneity) were violated. Multiple vehicle conditions were compared by Friedman's nonparametric analysis of variance. Specific comparisons were performed using Wilcoxon signed-rank tests or Sign tests. For all tests, a two-tailed p value less than 5 % was considered significant. For USV data, n= number of rats and for the electrochemical data, n= number of electrodes (see 'Voltammetric data analysis' below).

Experiment 1 Electrical stimulation of the medial forebrain bundle and FSCV recordings

Subjects

Please note: four of the rats used in this experiment were also used in Chapter 4

Eight experimentally naïve male Long-Evans rats (Charles River Laboratories, St. Constant, Quebec, Canada) were used, weighing 359-420 g at surgery. Subjects were housed two per cage before surgery and singly-housed after surgery. Home cages were kept in a temperature- and humidity-controlled colony room (20-22 °C, 50-60 %) with laboratory grade Sani-Chips bedding (Harlan Laboratories, Indianapolis, IN). Rats were kept on a reverse 12:12 h light/dark cycle, with lights off at 0730 hours. Behavioural testing took place between 0800 and 1300 hours. Food and water were available ad libitum, except during testing. Subjects were handled once daily for 5 min, for 4 days prior to the first experimental day. All procedures were approved by the McGill

Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care.

Electrochemical microsensor and reference electrode fabrication

Dopamine microsensors (i.e. working electrodes) and reference electrodes for chronic implantation were fabricated as previously described (Clark et al. 2010, Fortin et al. 2015). Briefly, a single carbon fiber (grade 34-700; Goodfellow Corporation, Caraopolis, PA, USA) was threaded through a fused silica shaft (Polymicro Technologies, Phoenix, AZ, USA) while submerged in 2-propanol. With a short length (approximately 17 mm) of carbon fiber protruding, two-component epoxy (Lepage Speed Set Epoxy™) was applied to one end and allowed to dry for 3-5 hrs. Next, silver epoxy (MG Chemicals, Allied Electronics, Fort Worth, TX, USA) was applied to the other end of the silica shaft in order to secure electrical contact between the carbon fiber and a gold-plated PCB socket connector (Newark Element 14 #23K7802, Chicago, IL, USA). The silver epoxy was left to dry over night, then coated with the two-component epoxy. The protruding length of carbon fiber was trimmed to 150-200 µm.

Reference electrodes were made using silver wire (A-M systems, bare: 0.010", coated: 0.013") cut into lengths of approximately 1 cm. A small drop of silver epoxy was applied to the open end of a nickel-plated brass pin (Newark Element 14 #82K7794, Chicago, IL, USA) and a single piece of uncoated silver wire was inserted. Once dry, the silver epoxy was covered with two-component epoxy and the protruding silver wire was trimmed to approximately 3 mm. The day before surgery, this wire was soaked in 10.2% sodium hypochlorite overnight, creating a silver/silver chloride (Ag/AgCl) surface interface.

Voltammetry surgery

All surgical procedures followed aseptic technique. Rats were anesthetized with isoflurane (5% induction, 2-2.5% maintenance, AErrane, Baxter). The scalp was shaved before the rat was placed in a stereotaxic frame. Polyvinyl alcohol (1% w/v, HypoTears, Novartis) was applied to the eyes, and a non-steroidal anti-inflammatory analgesic (carprofen, 5 mg/kg) and 0.9% sterile saline (2 mL) were administered subcutaneously (SC). The scalp was incised along the midline after topical application of Baxedin^{TM} antiseptic (0.05% w/v chlorhexidine gluconate + 4% v/v isopropyl alcohol) and local anesthetic (50:50 v/v mixture of 2% lidocaine and 0.5% bupivicaine). Skull holes were drilled and cleared of dura mater above the nucleus accumbens core (2.0 mm lateral and 1.7 mm rostral to bregma) and shell (0.9 mm lateral and 1.5 mm rostral to bregma); each rat received one microsensor implanted in each hemisphere (total of two microsensors per rat). Next, a skull hole was drilled in order to position the stimulating electrode (Plastics One, MS303/2-A/SP) above the medial forebrain bundle (MFB; 1.3 mm lateral and 4.6 mm caudal to bregma). Four to five additional holes were drilled at convenient locations for a reference electrode and 3-4 anchor screws. The reference electrode was lowered 3-4 mm from the skull surface (the entire length of silver wire) and one screw was secured to the skull; both were anchored with cranioplastic cement, leaving the stimulating electrode and microsensor holes exposed. The two microsensors were then attached to the voltammetric amplifier and lowered (0.2 mm/min) into the target recording regions (7.4 mm ventral of the brain surface for core and shell of nucleus accumbens). In order to determine the ideal location for placement of the stimulating electrode, the voltammetric waveform was applied at 10 Hz and catecholamines were monitored. Next, the stimulating electrode was lowered to 7.2 mm below dura mater and electrical stimulation (24 biphasic pulses, 60 Hz, ± 120 μA and 2 ms per phase) was applied via

an optically isolated, constant-current stimulator (A-M Systems, Sequim, Washington). If an evoked change in catecholamine concentration was not observed at the microsensor, the stimulating electrode was lowered in 0.3 mm steps until electrically-evoked catecholamine efflux was detected. The electrode was then lowered in 0.1 mm increments until catecholamine release was maximal. This usually occurred when the stimulating electrode was 8.4 or 8.7 mm ventral from the brain surface. If catecholamines were not detected at this point, the stimulating electrode was lowered to and kept at 8.7 mm. Finally, cranioplastic cement was applied to the exposed skull to secure the stimulating electrode, microsensors and the 2-3 additional screws. For post-operative pain management, subcutaneous carprofen was administered every 24 hours for four days.

Voltammetric data acquisition

For details of hardware and software, see Fortin et al. 2015. Waveform generation and data acquisition were carried out by using two input/output cards (NI PCI-6052E an NI PCI-6711, National Instruments, Quebec, Canada) and software written in LabVIEW (National Instruments). Signals from chronically implanted microsensors were forwarded to the data acquisition system via a head-mounted voltammetric amplifier (current-to-voltage converter) and an electrical commutator (Crist Instrument Co Inc, MD, USA) mounted in a custom-made Faraday cage. The voltammetric amplifier comprised an operational amplifier with a feedback resistor (R_f 5 ΩM) in parallel with a 6 pF capacitor to exclude high frequencies. To filter out operational amplifier noise, additional capacitors bridged each of the power sources (+15 V, −15 V) with ground. To promote a stable background current on test days, working electrodes were cycled with the voltammetric waveform at 60 Hz for 30 minutes and then at 10 Hz for 15

minutes (Moussy and Harrison 1994). It is not uncommon to observe a 200 mV shift in potential at reference electrodes over 2-5 days (Heien et al. 2005). This shift can be detected by the position of the Faradaic peaks within the background current. When observed, this was corrected by applying a 200 mV offset to the waveform (Heien et al., 2005). Voltammetric recordings consisted of a series of fast voltage scans, repeated at 100 ms intervals (i.e. 10 Hz). Each scan lasted 8.5 ms and comprised an ascending and descending linear (400 V/s) sweep between -0.4 and +1.3 V, applied to the microsensors in relation to the Ag/AgCl reference electrode. The potential was held at −0.4 V versus Ag/AgCl between scans. Electrical stimulation of the MFB was used to produce phasic catecholamine release. Each stimulation train (24 equally spaced biphasic pulses, 2 ms per phase) was spaced 5-6 minutes apart and the following eight stimulation parameters were used: 100µA and 120µA applied at 30 Hz, and 60 µA, 80 µA, 100 µA and 120 µA applied at 60 Hz. Voltammetric recordings were taken for 5 s before the electrical stimulation and for 10 s after. During this time, baseline USV recordings were taken for 55 s before each electrical stimulation, and for 55 s following the electrical stimulation.

Experimental protocol

Overview Rats were tested one month after surgery to allow for their recovery and the stabilization of electrodes (Polikov et al. 2005; Kozai et al. 2014). Four rats survived surgery and their head caps remained intact during the subsequent month, and were thus tested further. *In vivo* testing consisted of three parts: 1) Initial amphetamine screen, 2) Test day and 3) Characterization of the voltammetric signal.

Initial amphetamine screen A significant minority of rats emit few USVs in response to various stimuli (Schwarting et al. 2007). Therefore, rats underwent an initial amphetamine screen

comprising three test sessions, spaced 2 days apart (Wright et al., 2010; Chapter 2: Scardochio and Clarke, 2013). On each day, rats (n=4) were placed in test chambers for 20 min immediately after an IP injection of amphetamine (1 mg/kg, 1 mL/kg). Ultrasonic vocalizations emitted during the 12^{th}, 14^{th} and 16^{th} minute of the third session were counted, and all four rats were assessed as high callers relative to previous data (see Results).

Test day Testing resumed 20 days later. Rats were connected to the head-mounted voltammetric amplifier (see data acquisition above) and placed in the custom made test box consisting of Clear Plexiglas™ with laboratory grade Sani-Chips bedding. Half of the bedding was changed between each rat session. The electrical stimulation was not signaled by cues or demarcated by a specific point in time, and was thus unexpected.

Voltammetric data analysis

For the chemical identification of dopamine, current during a voltammetric scan was plotted against the applied potential and the background was subtracted (LabVIEW 7.1, National Instruments), yielding a background-subtracted cyclic voltammogram (CV). Verification of the putative DA signals was performed using the 'CV match' algorithm (Wightman et al. 1988; Heien et al. 2004; Clark et al. 2010) written in CV Analysis (LabView 7.1, National Instruments). Briefly, the background-subtracted CV was compared with a template voltammogram obtained from an earlier electrically-evoked (equally spaced biphasic pulses, 2 ms/phase, 60 Hz, 120 μA) *in vivo* recording, and a correlation coefficient was obtained. The phasic event was determined to be DAergic if the correlation coefficient was ≥0.75 (Heien et al. 2003; Cheer et al. 2004). In addition, DA transients were required to meet this criterion for at least two consecutive scans. Peak DA currents at the oxidation potential of DA (approximately

0.65 mV) were obtained from the background-subtracted CVs. Each recording electrode (two per rat) was treated as an independent unit for the following reasons: 1) the density of DA terminals at recording sites differ (Garris et al. 1994; Peters et al. 2000) 2) DA terminals within a given brain region differ in the extent to which they are autoinhibited (Moquin and Michael 2009) and 3) the existence of spatial and temporal heterogeneity of DA transmission in the ventral striatum where evoked responses are different in both amplitude and temporal profile (Wightman et al., 2007; Shu et al. 2013).

Characterization of in vivo voltammetric signals

We confirmed the dopaminergic nature of our *in vivo* voltammetry signals using three main criteria: electrochemical, pharmacological and anatomical.

Stimulated release The discharge of electrical current to DAergic cell bodies can act as a positive control for the detection of DA (Roitman et al. 2004). Here, stimulation of the MFB (24 equally spaced biphasic pulses, 2 ms/phase, see 'Voltammetric data acquisition' above for currents and frequencies) was used to detect DA release bilaterally in the NAcc of each rat.

Pharmacological validation DA and noradrenaline are indistinguishable by FSCV (Heien et al., 2003). Therefore, to further characterize the electrochemical signal, rats (n=3, one rat was removed due to electrode instability) were subjected to acute tests with systemically-administered drugs. The following drugs were used: the D2/D3 agonist (-)-quinpirole hydrochloride (Sigma Aldrich, Oakville, ON), the DAT blocker GBR12909 2HCl (NIMH Chemical Synthesis and Drug Supply Program), the D2/D3 antagonist raclopride, the α2-

adrenoreceptor antagonist yohimbine (Tocris Bioscience, Minneapolis, MN), the NET blocker desipramine hydrochloride (Sigma-RBI, St. Louis, MO) and D-amphetamine (Sigma-Aldrich, Poole, UK). Not all drugs were tested in each rat; see the Results section and Table S2 for details. All doses are expressed as the salt and all drugs were administered by the IP route (see Table S1 for drug details). All drugs were dissolved in 0.9% sterile saline except for: GBR12909 in dimethyl sulfoxide (DMSO) and yohimbine in distilled water. The timing and pH of each control (vehicle) injection matched that of the respective drug. Drugs were administered in a volume of 1 mL/kg except for GBR12909 (2 mL/kg).

Histological verification of recording sites DA is the predominant electroactive neurotransmitter within the NAcc and more specifically, in the lateral core (Garris et al., 1994) and rostral shell subregions (Park et al. 2010); thus, our microsensor targeted these regions. At the end of the experiment, rats were deeply anesthetized with IP ketamine (100 mg/kg) and xylazine (20 mg/kg) and an electrolytic lesion (+0.8 mA, 20 s, direct current) was made to facilitate histological identification. Rats were then transcardially perfused with saline and then a 10% aqueous formalin solution (Sigma, St.Louis, MO). The brain was then removed and flash-frozen at -50ºC with 2-methylbutane (Acros Organics, New Jersey, USA). Brains were sliced on a cryostat (25-µm coronal sections, -20 °C) and mounted on glass microscope slides. Sections were stained with cresyl violet to aid visualization of anatomical structures. When visible, the microsensor tip was localized by following the tract made by the fused-silica shaft.

Experiment 2 Optogenetics

Subjects

Seven 7th-generation heterozygote tyrosine hydroxylase TH:Cre males rats (Witten et al. 2011) from Dr. Shizgal's laboratory colony were used. These rats originated from a generous donation from Dr. Karl Deisseroth (Stanford University) and Dr. Ilana Witten (Princeton University), and were subsequently outbred with Long-Evans rats (Charles River Laboratories, St. Constant, Quebec, Canada). These transgenic rats express Cre recombinase under the control of the tyrosine hydroxylase (TH) promoter. Rats weighed 350-400g at surgery. Subjects were singly housed in a temperature and humidity-controlled colony room (20-22 °C, 50-60 %) and were kept on a reverse 12:12h light/dark cycle, with lights off at 0730 hours. Behavioural testing took place between 0800 and 1600 hours. Food and water were available ad libitum, except during testing. All procedures were carried out in accordance with the requirements of the Canadian Council on Animal Care and with the approval of the Concordia University Animal Research Ethics Committee.

Stereotaxic virus injections and optical fiber implantation

Rats were first anesthetized with a solution of ketamine (87 mg/kg, Bionicle, Bellville, Ontario) and xylazine (13 mg/kg, Bayer Inc., Toronto, Ontario), given IP in a volume of 1 mL/kg, followed by atropine sulfate (0.02-0.05 mg/kg, 1 m/kg, SC, Sandoz Canada Inc., Quebec) to reduce bronchial secretions. Polyvinyl alcohol (1% w/v, 'HypoTears' Novartis) was applied to the eyes, and penicillin procaine G (300 000 IU/mL, 0.3 mL, SC, Bimeda-MTC Animal Health Inc., Cambridge, Ontario) was used as a prophylactic antibiotic. Under isoflurane anesthesia (5% + O_2 for induction, 2-2.5% + O_2 for maintenance), the skull was exposed by scalpel incision and six small burr holes were drilled above target coordinates. For viral injections (AAV-DIO-ChR2-EYFP, University of North Carolina (UNC) Vector Core facility), a 28 gauge injector was

lowered into the VTA (anterior-posterior (AP) −5.4 and -6.2 mm; medial-lateral (ML) +/- 0.7 mm) and 0.5 µl of virus was infused at a rate of 0.1 µl/min at each of three dorsal-ventral (DV) coordinates (-8.2, -7.7 and -7.2 mm) for each of the two AP coordinates per hemisphere, for a total of 12 injections per brain. Virus was allowed to diffuse for 10 min between each injection. The total injection volume of virus was 6 µL (3 µL/hemisphere: three DV coordinates for each of the two AP coordinates). This virus-injection method produces widespread expression of ChR2, with partial spread into the pars compacta of the substantia nigra and to the contralateral VTA. Chronically implantable optic fibers with a 300 µm core were constructed following the methods described by (Sparta et al. 2012). Optical fibers were implanted bilaterally into the VTA at a 10° angle sloping laterally from the vertical (AP −5.8 mm; ML +/- 0.7 mm; DV −8.12 and -8.02 mm). Skull holes were covered with Gelfoam™ (Upjohn Company of Canada, Don Mills, Ontario) and the optical implants were anchored with a combination of skull screws and dental acrylic. Animals were given the opioid analgesic buprenorphine (0.05 mg/kg SC, 1 mL/kg, RB Pharmaceuticals Ltd., Berkshire, UK) and returned to their home cages for recovery. Behavioural manipulations began 5-7 weeks later to allow for viral construct expression.

Optical stimulation

In vivo optical stimulation of VTA neurons was achieved using a locally constructed 200 µm core optical fiber patchcord (Trujillo-Pisanty et al. 2015) attached to a chronically implanted fiber and leading to an optical rotary swivel with a FC input connector and an M3 output connector (Doric Lenses, Quebec, QC, Canada). The rotary swivel allowed the rats to move freely in the test cage. The rotary joint received input from a 473 nm laser (Shanghai Lasers, Shanghai, China) through a 125 µm optical fiber cable (62.2/125, Simplex, Thorlabs Inc.

Newton, New Jersey) secured via FC/PC adaptors. A pulse stimulator (A.M.P.I, Master-9, Jerusalem, Israel) was used to generate 1-s trains of 5-ms square pulses of blue light. Each rat received unilateral optogenetic stimulation of VTA neurons; the stimulating fiber (right or left hemisphere) and optimal stimulation frequency were determined for each rat (see 'Optical self-stimulation training' below). The laser was allowed to reach its operating temperature for 45-120 min before each training or test day. The laser power output through the patchcord was kept at 40 ± 2 mW across sessions and was verified every day.

Experimental protocol

Experiment 2 comprised the following four phases: 1) optogenetic self-stimulation training, 2) USV screen, 3) fixed interval training and 4) testing on multiple schedules.

Optogenetic self-stimulation training First, each rat (n=7) was screened in order to optimize the optical stimulation, by determining: (1) which optical probe (i.e. left or right) was more effective behaviourally, and (2) the optimal pulse frequency, defined as the lowest optical pulse frequency that supported a maximal rate of self-stimulation. To determine the most effective probe, a successive approximation procedure was used (Peterson 2004). Briefly, one optical implant was connected and rats were trained to lever press on an FR1 schedule for unilateral delivery of 1-s trains of 5-ms light pulses (473 mW, 60 Hz) into the VTA. Once the animal acquired the lever pressing behaviour, two 15-min FR1 trials began. Both trials began with the lever retracted, and were announced by a 10-s flashing light situated above the test box. Each stimulation was followed by a 2-s blackout period during which the lever was retracted, and the total number of stimulations obtained was counted. The contralateral optical probe was tested the following day in the same way. The probe that supported the most pressing was considered most effective and was used for the rest of the experiment. In some rats, both probes produced similar behavioural

outputs. When this happened, both optical probes were retested on a third day and the most effective one from that session was retained. Next, to determine the optimal pulse frequency, rats were permitted to lever-press for unilateral optical stimulation of the VTA, in a 2-s cumulative handling time schedule (Breton et al. 2009). In this schedule, rats earned one train of VTA stimulation (1-s duration, 5-ms light pulses) for every 2 s of hold-down (accumulated within and across different hold-downs). Each trial was announced by a 10-s flashing light. At 8 s after the onset of the flashing light, a noncontingent priming stimulation was delivered which matched the pulse-frequency of the contingent stimulation offered in that trial. The pulse frequency of each priming and response-contingent stimulation was systematically decremented across ten 2-min trials to obtain a sigmoidal pulse-frequency vs. response rate curve. The pulse frequencies varied across subjects, but typically ranged from 2 Hz (lower asymptote) to 60 Hz (higher asymptote). Between two and five sessions were required to construct a reliable frequency-response curve. A 2-s blackout period followed each contingent stimulation and the lever was retracted during this time. The lowest pulse frequency which yielded maximal responding was considered optimal. In all subsequent testing, the optimal pulse frequency were held constant for a given rat (Table S3).

USV screen Near the end of optical self-stimulation training, rats were screened for USV emission in a single session. Acoustic recordings began when rats were removed from their home cage and continued for 2-5 minutes during optical self-stimulation training in their test box. Only rats that emitted USVs (>5 calls/min) during this time were retained in the experiment (n=5 of 7).

Fixed-interval (FI) training Over the course of 4-7 days (depending on the rat), animals were trained to lever press for progressively longer fixed interval (FI) schedules, starting with FI-01 (1

s) and ending with FI-20. Rats did not advance to the next schedule until they were pressing reliably at that stage. Every day, each rat completed 3 to 12 sessions, each lasting 20 min. Rats began test days once they were reliably pressing under FI-20.

Testing on multiple schedules Over the course of 8 consecutive days, each rat was tested on four different 20-s schedules of optogenetic presentation, i.e. fixed and variable interval (FI-20, VI-20), and fixed and variable time (FT-20, VT-20). Each schedule was tested in two test sessions (1 session per day) lasting 2 hours. Schedules were tested in the following order FI-20, FT-20, VI-20 and VT-20. Under the FI and FT schedules, the stimulation was *expected*, insofar as it was delivered (FT) or became available (FI) every 20 s. Under VI and VT schedules, the stimulation was always *unexpected*, because the time between consecutive stimulations was sampled from a 1.2 second lagged exponential distribution. Lever presses were recorded using software written in LabVIEW (National Instruments). Under fixed and variable *interval* schedules, the lever was always extended and a single lever-press was required for optical stimulation of the VTA (i.e. the rat determined the stimulation time once the minimum interval had elapsed). For fixed and variable *time* schedules, the stimulation was delivered non-contingently, and no lever was present. The interval length (20 s) and the test session duration (2 hours) were selected based on previous work (Ludvig et al. 2007; Chehayeb 2007). USVs were recorded for the whole session. No discrete cues (e.g. light or tone) were present and testing was performed in a dark room illuminated only with a red light. Since the rats virtually stopped calling within 30 min (see Results), therefore only the first 30 min of calls and lever-pressing behaviour were analyzed.

Histology (not completed)

The rats used in this experiment are currently being used in another experiment. Histology will be performed at a late date using the following protocol.

Histology was performed to colocalize yellow fluorescent protein (YFP) and tyrosine hydroxylase (TH), so as to confirm virus expression (YFP) in DAergic neurons (TH). Hence, upon completion of *in vivo* experiments, rats were transcardially perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS). Brains were removed and post-fixed in a 4% paraformaldehyde/30% sucrose solution for two days, and kept frozen at -20 °C thereafter. 40 µm sections were mounted on Superfrost™ microscope slides (Thermo Fisher Scientific, Ottawa, Ontario), washed with PBST (phosphate buffered saline with Triton: 0.1M PBS + 2 g/L bovine serum albumin + 2 mL/L Triton X) for 20 min, and incubated in normal donkey serum (Sigma Aldrich, Oakville, ON) in PBT for 30 min. To assess the spread and selectivity of expression of the viral construct, anti-TH and anti-GFP immunohistochemistry was performed. Slices were exposed for 24 hrs to the following primary antibody mixture: mouse anti-GFP (1:1500, Invitrogen, #A11120) and rabbit anti-TH (1:500, Fisher, #AB152MI). Slices were washed with PBT (3 x 5 min) and incubated with 2% normal donkey serum in PBS for 10 min. Tissues were incubated for 2 hrs at room temperature with secondary antibodies: donkey anti-mouse AlexaFluor 488 (1:200, Jackson Immuno, West Grove, PA, USA) and donkey anti-rabbit AlexaFluor 594 (1:200, Jackson Immuno), for YFP and TH respectively. Sections were subsequently imaged (10x) on an Olympus FV10i-LIV confocal microscope to show colocalization of TH and the ChR2-YFP construct.

Results

Experiment 1: MFB stimulation and rat ultrasonic vocalizations

Initial amphetamine screen

The median 50-kHz call rate on the third day of the AMPH screen was 76 calls per min (range 53-102, n=4 rats). Based on previous studies (Wright et al., 2010; Ahrens et al. 2013; Chapter 2: Scardochio and Clarke, 2013), all the rats would be classified as 'high callers' (i.e. above-average call rate). One rat was removed from the remaining analysis because its electrical connection failed intermittently (hence, n= 3 rats).

Ultrasonic vocalizations following electrical stimulation of the MFB

Electrical stimulation of the MFB significantly increased 50-kHz call rate (Sign test, $p<0.002$, Fig. 1). Calls were evoked promptly, starting approx. 1 s from the start of each stimulation train (Fig. 2). The main call subtypes emitted following MFB stimulation were trill, flat/trill combo and flat, which accounted for 42%, 27% and 13% of all calls, respectively (Fig. S1). In one rat, more intense stimulation parameters were tested (60 Hz, 140 µA), producing 22-kHz as well as 50-kHz calls; therefore, these stimulation parameters were not tested with the other rats.

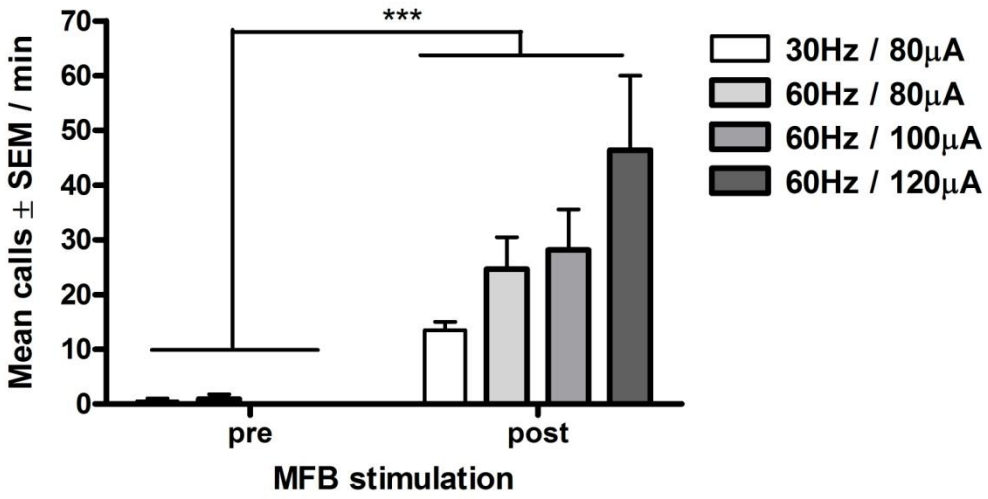

Fig. 1 Electrical stimulation of the medial forebrain bundle (MFB) and 50-kHz call rate (Experiment 1). Each rat (n=3) was tested under each stimulation condition. Calls after MFB stimulation (post, 0-55 s following stimulation onset) were significantly greater than calls before MFB stimulation (pre, 0-55 s before stimulation). Each stimulation train was spaced 5-6 min apart. ***$p<0.002$ pre vs. post (Sign test, n= 12 i.e. 4 stimulation parameters x 3 rats)

Phasic DA release in the nucleus accumbens following electrical stimulation of the MFB

Data presentation is restricted to the five electrodes confirmed to be located in the nucleus accumbens and recording phasic DA release; only one electrode was excluded (see Characterization of in vivo voltammetric signals below).

Unilateral electrical stimulation of the MFB evoked phasic dopamine release in the NAcc (Fig. 2). The evoked release was bilateral (n=2 rats, data not shown). Mean peak DA currents increased with increasing applied current, for both stimulation frequencies, i.e. 30Hz (Wilcoxon, $p<0.05$) and 60Hz (Friedman, $p=0.002$) (Fig. 3). MFB stimulation rapidly increased the call rate (Fig. 2a) and the DA signal (Fig. 2b), with both effects appearing within approx. 1 s of stimulation onset. In contrast, the vocalization response far outlasted the transient DA signal

increase. A subset of stimulations (32% of total stimulations) produced an increase in phasic DA in the NAcc without evoking an increase in call rate (for a summary of results for each rat under each stimulation parameter, see Table S4).

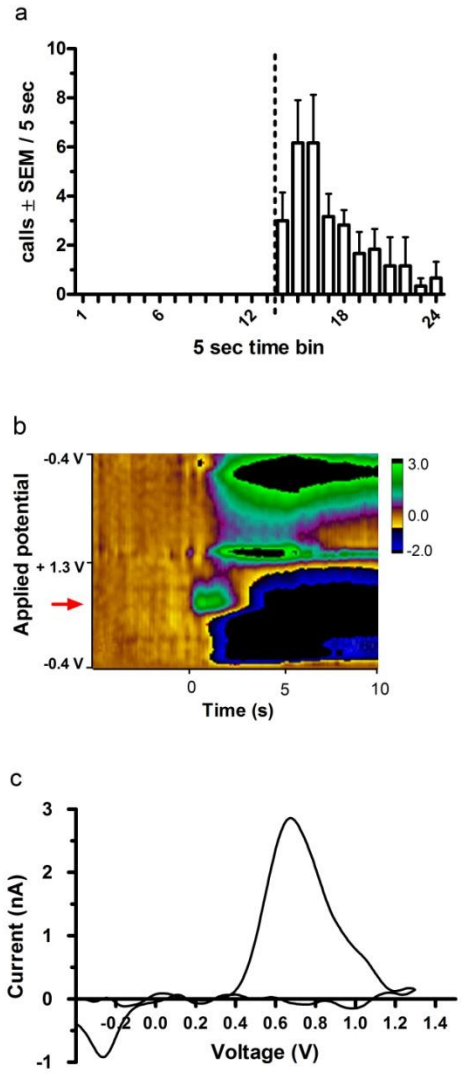

Fig. 2 Time course of 50-kHz call emission and phasic DA release following electrical stimulation of the medial forebrain bundle (MFB). Stimulated DA release in the nucleus accumbens (NAcc) was timed-locked to MFB stimulation (60 Hz, 24 pulses, biphasic, 2ms/phase, 100µA) and the onset of USV emission. Panel **a** shows an increase in call rate

following MFB stimulation. The 1-s stimulation started at 0 s. Panel **b** is a color plot from a representative rat, showing changes in DA current (green color) in relation to applied potential (y-axis) and time (x axis), with onset of MFB stimulation occurring at t=0 s, at the oxidation potential of dopamine (red arrow), i.e. approx. 0.65V (vs. Ag/AgCl reference). Panel **c** shows the corresponding background-subtracted cyclic voltammogram from the same rat at the point of peak DA current seen in panel b.

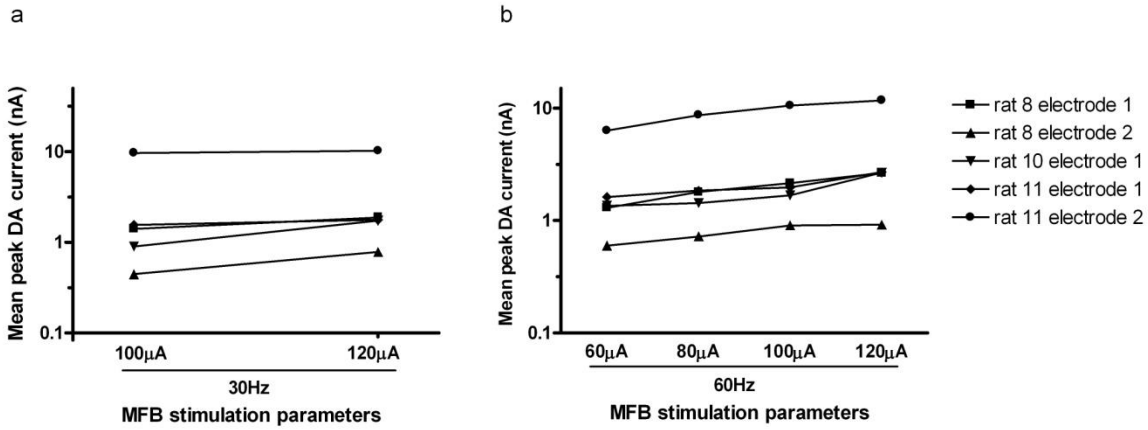

Fig. 3 Mean peak DA current for each electrode (n=5 electrodes) across various stimulation parameters. At both frequencies tested (30 Hz - panel **a;** 60 Hz – panel **b**), peak DA current increased with increasing current (respectively: Wilcoxon, $p<0.05$; Friedman, $p=0.002$).

Characterization of in vivo voltammetric signals

Electrically-evoked release Following stimulation of MFB fibers, DA was detected at almost all recording sites, as revealed by background-subtracted CVs (Fig. 2b, c). The exception was microsensor (i.e. electrode) #2 from rat 10, which was excluded from all analyses.

Pharmacological verification The peak DA current evoked by electrical stimulation increased significantly following an acute administration of: the indirect DA/NA agonist amphetamine

(paired t-test $p<0.05$); the D2 antagonist raclopride (paired t-test, $p<0.05$); and the DAT blocker GBR12909 (Fig. S2, paired t-test, $p<0.02$). The three other pharmacological conditions could not be assessed statistically because each drug was tested in a single rat; this is because two of the three rats died prematurely, one day after an injection of desipramine/yohimbine, and the other under anesthesia while its headcap was being repaired. See Table S2 for details of completed drug conditions for each rat.

Histology Five electrode tips were confirmed to lie within the NAcc (Fig. S3). One electrode location could not be verified and the corresponding data are not presented; this was the same electrode (#2 of rat 10) where electrically-evoked catecholamine release was not obtained.

Experiment 2: Optogenetic stimulation of VTA dopaminergic neurons

One of the five rats in this experiment did not emit any USVs on test days and was thus excluded from the analysis. The remaining four rats worked to obtain trains of optogenetic stimulation on both FI-20 and VI-20 test schedules. As anticipated, responding was schedule-dependent. Thus, rats pressed faster towards the end of the 20-s fixed interval, whereas they maintained a steady response rate during the 20-s variable interval in which the timing of the stimulation was unpredictable (Fig. 4).

Within the first 30 min of the test session, response rates were maintained (Fig. 5a-b) whereas call rates steadily declined (Fig. 5d-f). Over the rest of the 2-h session, lever-press rates tended to increase (Fig. 5c), but 50-kHz calling remained virtually undetectable (mean 1 ± 0.9 calls/min).

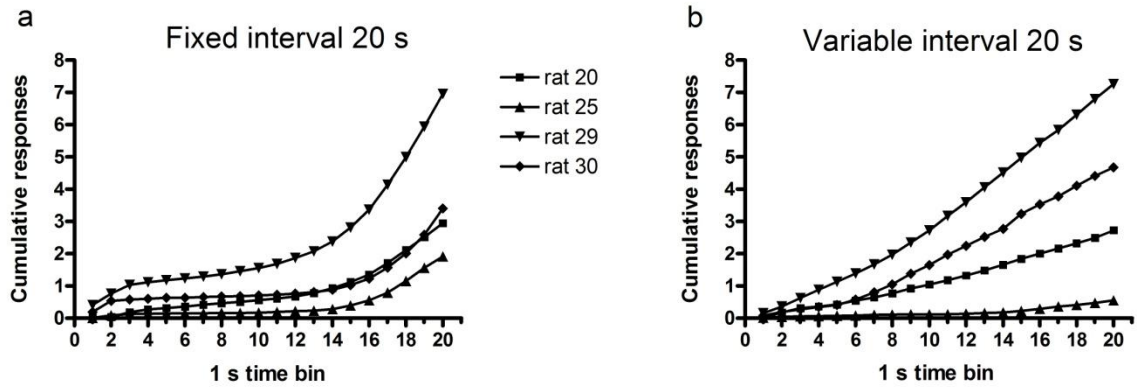

Fig. 4 Cumulative lever responses for each rat (n=4) as a function of time since the last optogenetic stimulation. Panel **a** shows lever responses increasing as the next stimulation opportunity approached (at 20s). Panel **b** shows constant-rate lever pressing throughout the session, where timing of the next stimulation opportunity was unknown (times randomly selected from lagged exponential distribution with mean 20s).

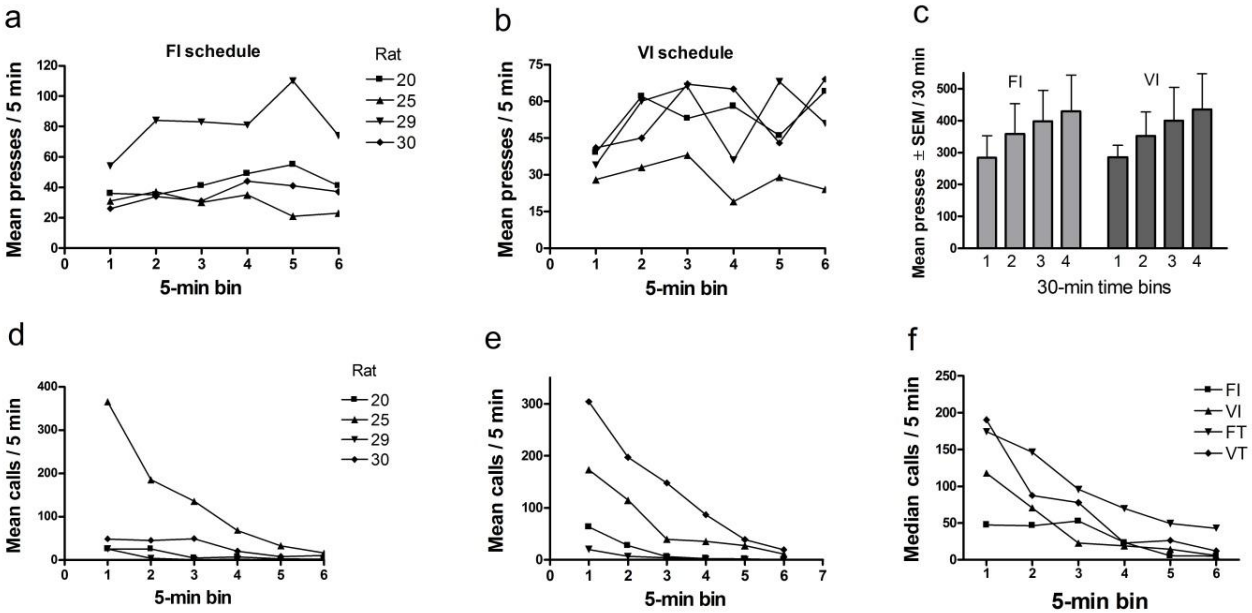

Fig. 5 Mean lever presses and mean calls for each schedule (n=4 rats). Panels **a** and **b** show that lever press rates under both schedules remained stable across successive 5-min time bins within the first 30 min of the session. Panel **c** shows that response rates tended to increase across the 2 hour session. Panels **d** and **e** show mean calls across FI and VI reinforcement schedules, decreasing over the first 30 minutes of the session for each rat. Panel **f** shows that the median rate decreased over the first 30 minutes for all schedules. FI: fixed interval, VI: variable interval, FT: fixed interval, VT: variable interval. All schedules are 20 s.

USV emission: call numbers, categories and timing with optogenetic stimulation

Rats called at a broadly similar rate before and after each stimulation (Fig. 6). Thus, call rates occurring in the 10 s either side of the stimulation did not differ significantly on any of the four schedules (Wilcoxon test, NS for each schedule). However, rats called less during the 1-s optogenetic stimulation itself under all schedules except fixed time (Fig. 6). Overall, call rates were significantly higher on 'time' schedules than on 'interval' schedules (Fig. 7b, Sign test, $p<0.02$).

Call profiles (i.e. percentage prevalence of 14 call subtypes and two call categories) are shown in Fig. 6 (insets) and Fig. S4. These profiles were comparable across the four schedules, and also between the 10-s periods before and after each stimulation (Friedman, NS). Overall, the most prevalent call subtypes were trills, flats, and flat-trills accounting for 49%, 15% and 10% respectively (Table S5). Further details are given in Table S6.

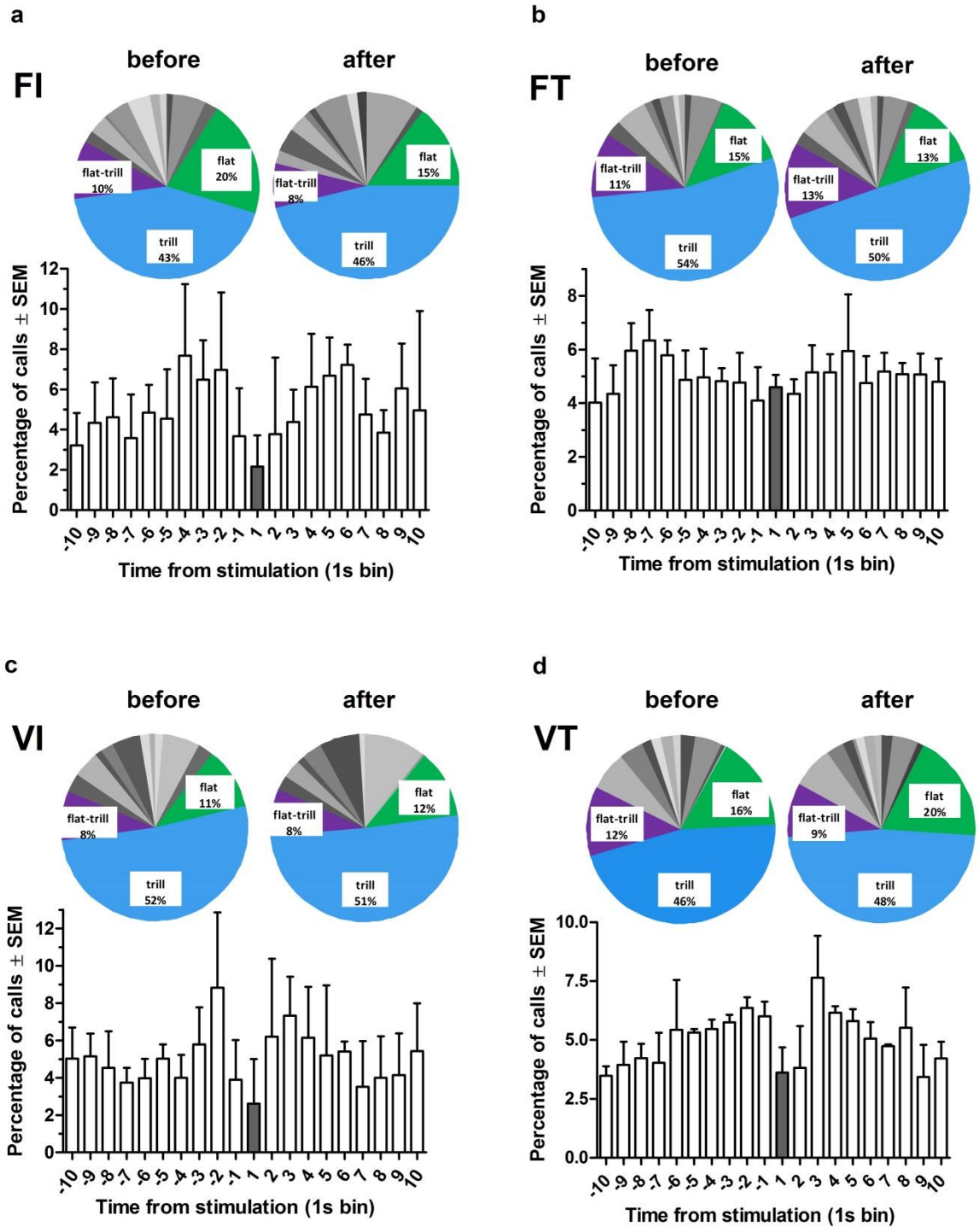

Fig. 6 Percentage of total calls and call subtypes under each reinforcement schedule for all rats (n=4), as a function of time from stimulation. Time bin '1' represents the time at which the VTA optogenetic stimulation occurred. Pie charts show percentages of calls (14 subtypes and 2

categories) before (-10 to -1) and after (1 to 10) the stimulation. The most common calls are labeled (for details of call subtypes see Table S5). FI: fixed interval, FT: fixed time, VI: variable interval, VT: variable time. All schedules are 20 s.

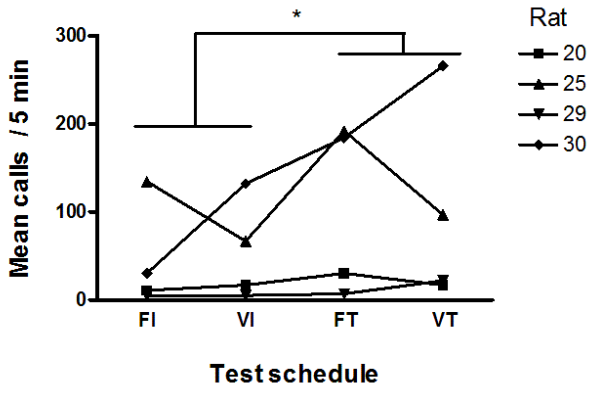

Fig. 7 Mean calls emitted during each reinforcement schedule. Mean number of calls emitted under each schedule for each rat (n= 2 sessions), showing a significant increase in call number when the optogenetic stimulation was non-contingent (VT and FT schedules) *vs.* contingent on a lever-press (FI and VI schedules). FI: fixed interval, VI: variable interval, VT: variable time, FT: fixed time. Sign-test *$p<0.02$ (n=8 i.e. 4 rats x 2 schedules)

Discussion

The present study provides two main novel findings. First, unexpected electrical stimulation of the MFB elicited both phasic DA release in the NAcc (lasting approx. 2 sec) and a longer-lasting increase in 50-kHz vocalizations. Second, neurochemically-selective optogenetic stimulation of VTA DA neurons, although reinforcing, did not consistently elicit 50-kHz calls.

Electrical stimulation of the MFB produced concurrent USV emission and phasic DA release in the NAcc

In Experiment 1, unexpected electrical stimulation of the MFB elicited 50-kHz vocalizations with minimal post-stimulation delay. Short-latency, electrically-evoked 50-kHz calling was previously reported in association with VTA and six other brain regions (Burgdorf et al., 2007); however, the relevant data were not presented for individual brain regions. The same authors reported quite different results in an earlier study, which featured non-contingent (FT-20 schedule) electrical stimulation of the VTA (Burgdorf et al., 2000). Here, 50-kHz calls occurred, not in immediate response to the stimulation, but rather in apparent anticipation of it. At least three factors could contribute to these differences in outcome between these three studies. First, Burgdorf et al. (2000) used a heterodyne bat detector which would have detected only the subset of 50-kHz calls falling within its narrow frequency band. Second, our stimulation parameters differ considerably from those used in the two earlier reports. Third, we have directly detected the resultant DA release whereas in the earlier studies, evoked DA release was inferred from the observed behaviour (i.e. reinforced responding).

Vocalizations co-occurred with electrically-evoked phasic DA release in the NAcc, but with exceptions. Notably, during a significant minority of trials (32%), electrical stimulation elicited phasic DA release but did not elicit any 50-kHz USVs. During these trials, peak DA release was not noticeably lower, suggesting that calls are not simply triggered when a specific threshold of peak concentration is achieved. In this context, it is worth noting that electrical stimulation of the MFB releases many other transmitters, including 5-HT, acetylcholine, glutamate and

noradrenaline, which all appear to modulate the emission of USVs (Brudzynski and Bihari, 1990; Wintik and Brudzynski, 2001; Wright et al., 2012; Wohr et al., 2015) .

Disappearance of USVs despite continued optical stimulation

Fifty-kHz calls gradually disappeared over the first 30 minutes of the 2-hour session, despite continuing optogenetic stimulation of the VTA. There are several possible explanations for this divergence. A trivial explanation would be that the larynx, which generates USVs (Johnson et al. 2010), became over-used and no longer capable of producing calls. However, this is most unlikely since adult rats can sustain high 50-kHz call rates (>50 calls/min) for at least an hour after acute amphetamine administration (unpublished). A second possibility is that with repeated optogenetic stimulation, phasic DA transmission is no longer sufficiently enhanced to support USV production. Three sets of observations support this notion. First, intense optogenetic stimulation can partially desensitize channelrhodopsin-2 (Bamann et al. 2008; Lorenz-Fonfria and Heberle 2014). Second, intracranial self-stimulation studies have shown that intense neuronal activation can deplete the pool of releasable DA (Garris et al. 1999; Kilpatrick et al. 2000; Park et al. 2013). However, against this, phasic DA release was maintained over 2 hours during electrical stimulation of the MFB applied at shorter stimulation intervals (10 s) (Cheer et al. 2005) than used in our experiment. A third mechanism by which DA transmission might be reduced is the desensitization of postsynaptic DA receptors, as has been observed in studies using electrical stimulation or pharmacological manipulations (Beaulieu and Gainetdinov 2011). This, too, appears unlikely given the persistence of optogenetic self-stimulation, a behaviour which depends critically on phasic DA release (Bass et al. 2010; Bass et al. 2013; McCutcheon et

al. 2014) and on functional D1 and D2 receptors in the nucleus accumbens (Steinberg et al. 2013; Steinberg et al. 2014).

A final possibility that we considered is that by 30 minutes into the session, the self-stimulation behaviour had become habitual and thus insensitive to the outcome, i.e. evoked DA release. This question was addressed experimentally, by testing an additional group of rats (n=4) in extinction (Fig. S4). We found that when the optogenetic light source was turned off after one hour of self-stimulation, subjects abruptly stopped lever pressing, and only resumed responding when the stimulation was reinstated. Importantly, this experiment used more intense optogenetic stimulation parameters than in Experiment 2 (Fig. S4 legend), which further suggests that prolonged stimulation in Experiment 2 did not lose its effectiveness in activating DAergic neurons (i.e. ChR2 not desensitized).

High USV call rate during the first 30 minutes of optogenetic stimulation of VTA DAergic neurons

Early in the 2-hour session, optogenetic stimulation was associated with high rates of 50-kHz calling (approx. 30 calls/min) with a marked decline across the first 30 minutes, across all four schedules of delivery. However, during the 1-second optogenetic stimulation itself, call rates tended to decrease even though extracellular DA concentrations were rapidly rising, based on reports from anesthetized and freely-moving rats (Bass et al., 2010; MP Cossette and P Shizgal unpublished).

Call profile following optogenetic stimulation of VTA

Typically, 50-kHz vocalizations are analyzed as a single group of calls or are dichotomized as frequency-modulated and flat subtypes (Portfors, 2007). However, 50-kHz calls are far more heterogeneous, with at least 14 identifiable subtypes (Wright et al., 2010). During optogenetic stimulation sessions, the most commonly emitted call was the trill subtype. Among 50-kHz call subtypes, it is the trill call that is preferentially suppressed by DA-depleting lesions and DA antagonist administration (Ciucci et al. 2009; Wright et al., 2013), two manipulations which would be expected to reduce both tonic and phasic DA transmission. The preponderance of trill calls in opto-stimulation sessions suggests that this call subtype is promoted by phasic DA release.

Frequency-modulated calls, and trills in particular, occur in situations where positive affect putatively occurs. For example, these calls are especially abundant in the following experimental situations: (1) acute systemic administration of psychostimulants (Ahrens et al. 2009; Wright et al., 2012; Chapter 2: Scardochio and Clarke, 2013; Simola et al. 2014), (2) anticipated delivery of food or drug delivery (Buck et al. 2014a; Buck et al. 2014b; Opiol et al. 2015), (3) rough-and-tumble play (Knutson et al. 1998), and (4) sexual behaviour (Snoeren and Agmo 2014). Since we have shown that reinforced lever pressing can be dissociated from USV emission, it appears that trill calls, insofar as they track positive affect, do so independently of positive reinforcement.

Effects of different schedules of optogenetic stimulation of the VTA on USV call rates and profiles

Call rate and call subtypes under 'fixed' (i.e. expected stimulation) and 'variable' (i.e. unexpected stimulation) reinforcement schedules did not differ detectably. This suggests that 50-kHz calls do not report reward prediction error, one of the reported functions of phasic DA transmission (Schultz 1998; Glimcher 2011). Of the four schedules used in Experiment 2, the FI and VI schedules featured *response-contingent* reinforcement while the FT and VT schedules involved *non-contingent* ("free") reinforcement. Call rates were significantly higher following non-contingent opto-stimulation of VTA DAergic neurons. In line with this, rats have been shown to prefer rewards that require low effort (Gan et al. 2010), and the availability of such rewards is concurrently reflected by phasic dopamine release in the NAcc (Day et al. 2010) Furthermore, phasic DA has been shown to scale with rats' preferred choices (Saddoris et al. 2015). The link between preferred rewards (i.e. low effort) and DA release, and our observed increase in USV emission, suggests that USV call *rate* may potentially indicate preferences between different rewards.

Limitations

Pharmacology In Experiment 2, several pharmacological verification tests were planned in order to confirm the neurochemical identity of the FSCV signal evoked by electrical stimulation. However, as detailed in Results, three drug tests were curtailed because of unforeseen adverse events. For example, the combination of yohimbine and desipramine appeared highly toxic, even though the same dose combination has been used previously without apparent ill effect (Park et al., 2010). Despite the restricted pharmacological testing, two additional factors indicate that our

FSCV signal would have been minimally contaminated by extracellular NA: first, our recording electrodes were located in parts of the NAcc that receive little or no NA innervation (Park et al., 2010) and second, the recording electrodes used here are reportedly more sensitive to DA than NA (Clark et al., 2010).

VTA efferents Although we did not attempt to measure phasic DA release during optogenetic stimulation of the VTA (Experiment 2), it is very likely to have occurred for three main reasons. First, previous *in vitro* and *in vivo* studies have shown that optogenetic stimulation of VTA DAergic neurons reliably evokes phasic DA release, in downstream targets such as the NAcc and dorsal striatum (Bass et al., 2010; Chiu et al., 2014). Second, the virus transfection was restricted to VTA DAergic neurons. Third, our unpublished recordings (MP Cossette and P Shizgal) have confirmed that phasic DA release occurs in the NAcc following optogenetic stimulation of the VTA, using identical stimulation parameters.

Optogenetic test day order In Experiment 2, the reinforcement schedules were tested in a fixed order, as follows: fixed-interval, fixed-time, variable-interval and variable-time. This particular sequence was chosen so as to limit the number of training sessions after the acquisition of FR1 responding. Testing order, however, would not have acted as a significant confounder given that all four schedules dissociated persistent lever-pressing behaviour from declining USV emission.

Conclusion

We have confirmed that immediately following electrical stimulation of the MFB, rats start to emit 50-kHz calls. The onset of these vocalizations coincided with phasic DA release in the

NAcc. Optical stimulation of VTA DAergic neurons was associated with high 50-kHz call rates and a trill-rich call profile, as occurs after systemic administration of psychostimulants. However, because optogenetic stimulation of VTA DAergic neurons did not produce sustained calling, experimentally-induced phasic DA release in the NAcc does not appear a *sufficient* stimulus to induce 50-kHz call emission.

Connecting Text 2

In the previous chapter, we tested our hypothesis that 50-kHz vocalizations are driven by phasic DA release. We found that stimulation of dopaminergic (DAergic) neurons led to the emission of 50-kHz calls and phasic DA in the nucleus accumbens in a time-locked manner. We next showed that *selective* activation of VTA DAergic neurons produced a high 50-kHz call rate. However, calls were not time-locked with the transient stimulation of DAergic neurons. Furthermore, calls decreased across the first 30 minutes of the session despite persistent stimulation. In the next chapter, we examined the potential contribution of phasic DA release to the emission of 50-kHz calls emitted following amphetamine administration. First, we used selective DA receptor agonists to tonically activate post-synaptic receptors before the administration of amphetamine. Next, we completed an amphetamine dose-response experiment during which we recorded both USVs and phasic DA release (using fast-scan cyclic voltammetry) in freely moving rats.

CHAPTER 4: Investigation of the role of phasic dopamine release in amphetamine-induced rat ultrasonic vocalizations

Tina Scardochio, Stefan G Sandberg, Paul E. Phillips, Paul B.S. Clarke

Abstract

Rationale Systemic administration of amphetamine (AMPH), an indirect dopaminergic and noradrenergic agonist, reliably increases adult rat 50-kHz call rate. This stimulatory effect has not been mimicked, however, by several other drugs that promote dopaminergic or noradrenergic transmission, i.e. direct-acting receptor agonists and transporter blockers. We therefore hypothesize that AMPH's ability to stimulate 50-kHz calling results from its reported ability to increase the frequency and amplitude of phasic dopamine (DA) release events.

Objective To test whether: 1) AMPH-induced calling is inhibited by co-administration of DA receptor subtype-selective agonists, and 2) 50-kHz vocalizations occur simultaneously with transient DA release events.

Methods Ultrasonic vocalizations (USVs) were recorded in adult male rats after acute systemic of dopaminergic drugs. In both experiments, subjects were initially screened with AMPH (1 mg/kg IP), primarily to exclude low callers. In Experiment 1, adult male rats were tested with: AMPH (dose-response: 0, 0.1, 0.3, 0.6, 1.6 and 4 mg/kg IP), and subsequently AMPH (0.6 mg/kg IP) given alone or with single doses of the agonists A68930 (D1-like), quinpirole (D2-like) or PD 128907 (D3). Experiment 2 comprised an AMPH dose-response study (same doses as Experiment 1) in which phasic DA release events were concurrently monitored in the nucleus accumbens (NAcc) using fast-scan cyclic voltammetry.

Results In both experiments, intermediate doses of AMPH strongly increased 50-kHz call rates, with the trill call subtype becoming especially prevalent. In contrast, in Experiment 1, the D1-

like agonist A68930 and the D2/D3 selective agonist quinpirole both decreased AMPH-induced calling; quinpirole also inhibited calling when given alone. The D3-selective agonist PD 128907 had no detectable effect. In Experiment 2, AMPH not only stimulated 50-kHz calling but also increased the amplitude of phasic DA events in the NAcc, without affecting their frequency or duration. However, most USVs (>95%) occurred in the absence of phasic DA events.

Conclusions Our pharmacological findings are consistent with the hypothesis that AMPH-induced calling is mediated by phasic DA release in the brain. However, phasic DA release events occurring in the NAcc appear neither sufficient nor necessary for 50-kHz call emission.

Introduction

Adult rat ultrasonic vocalizations (USVs) are commonly divided into two main categories, 22-kHz and 50-kHz, respectively associated with negative and positive affect (Knutson et al. 2002; Brudzynski 2013). The 50-kHz call type is heterogeneous, with at least 14 distinct subtypes (Wright et al. 2010). The emission of 50-kHz calls depends on multiple neurotransmitter systems (Fu and Brudzynski 1994; Brudzynski and Barnabi 1996; Wintink and Brudzynski 2001; Arnold et al. 2010; Wright et al. 2012; Sadananda et al. 2012; Wright et al. 2013; Chapter 2: Scardochio and Clarke 2013; Wohr et al. 2015), among which the mesolimbic dopaminergic (DAergic) system has been extensively implicated (Cagiano et al. 1989; Burgdorf et al. 2001; Wintink and Brudzynski, 2001; Burgdorf et al. 2007; Ciucci et al. 2009; Bialy et al. 2010; Brudzynski et al. 2012; Wright et al., 2013; Chapter 2: Scardochio and Clarke, 2013; Willuhn et al. 2014). For example, both spontaneous and drug-induced calls are strongly inhibited by manipulations that reduce DAergic transmission, i.e. administration of DA receptor antagonists (Thompson et al., 2006; Wright et al., 2013; Chapter 2: Scardochio and Clarke, 2013) or 6-hydroxydopamine and electrolytic lesions of the lateral hypothalamus or ventral tegmental area (VTA) (Burgdorf et al., 2007).

Fifty-kHz calls, especially the trill subtype, are reliably elicited by systemic administration of psychostimulants including d-amphetamine (Burgdorf et al., 2001; Thompson et al. 2006; Ahrens et al. 2009; Wright et al. 2010a; Wright et al., 2013; Chapter 2: Scardochio and Clarke, 2013; Simola et al. 2013; Simola et al. 2014; Wohr et al., 2015). In marked contrast, direct DA agonists *inhibited* 50-kHz calling (Chapter 2: Scardochio and Clarke, 2013), and in addition, the DA and noradrenaline transporter blockers, given alone or together, failed to elicit calls (Wright et al., 2012).

How to explain these disparate findings? Among DAergic drugs, psychostimulants appear unique in their ability to increase phasic DA release (Heien et al. 2005; Cheer et al. 2007; Owesson-White et al. 2009; Daberkow et al. 2013; Covey et al. 2013; Cameron et al. 2014). In the present study, we hypothesized that phasic DA release promotes the emission of *amphetamine*-induced calls. As noted above, both DAergic antagonists and agonists decrease 50-kHz calling after systemic administration (Chapter 2: Scardochio and Clarke, 2013). These two drug classes have opposite effects on tonic DA transmission, but they share the ability to blunt the postsynaptic impact of phasic DA release events. This common effect arises because DA agonists tonically activate postsynaptic DA receptors, thereby masking the intermittent receptor activation that would normally follow phasic DA release. Therefore, AMPH-induced 50-kHz call emission, if driven by phasic DA release, should be inhibited by a DA agonist. In Experiment 1, we tested the effects of co-administering AMPH with DA receptor subtype-selective DAergic agonists. Subsequently, in Experiment 2, we simultaneously recorded AMPH-induced USVs and phasic DA release in freely-moving animals; this approach allowed us to investigate how USV occurrence might be related to the timing, frequency, duration and amplitude of transient DA release events.

Methods

Subjects

Subjects were 32 (Experiment 1) and 15 (Experiment 2) experimentally naïve male Long-Evans rats (Charles River Laboratories, St. Constant, Quebec, Canada). Rats weighed 236-275 g at the start of the experiment (Experiment 1) and 359-530 g at surgery (Experiment 2). Subjects were housed two per cage in a temperature and humidity-controlled colony room (20-22 °C, 50-60 %). Home cage bedding consisted of laboratory grade Sani-Chips (Harlan Laboratories, Indianapolis, IN). Rats were kept on a reverse 12:12h light/dark cycle, with lights off at 0730 hours. Behavioural testing took place between 0800 and 1300 hours. Food and water were available ad libitum, except during testing. Subjects were handled once daily for 2-5 min, for 2-4 days prior to the first experimental day. All procedures were approved by the McGill Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care.

Acoustic data acquisition and identification of ultrasonic vocalizations

The testing procedure and acoustic analysis were as previously described (Wright et al., 2010). For Experiment 1, clear Plexiglas™ experimental boxes (ENV-007CT, Med Associates, St. Albans, VT) were used. Each box was enclosed in a separate melamine compartment lined with sound-attenuating acoustic foam (Primacoustic®, Port Coquitlam, BC). For Experiment 2, the testing box was enclosed in a Faraday cage (made in-house) lined with sound attenuating acoustic foam. Ultrasound condenser microphones (CM16/CMPA, Avisoft Bioacoustics, Berlin, Germany) were located at the top center of each experimental box, 25-40 cm from the rat. Microphone signals were delivered to an UltraSoundGate 416H data acquisition device (Avisoft Bioacoustics) with a sampling rate of 250 kHz and 16-bit resolution. Avisoft SASLab Pro

software (version 5.1.14, Avisoft Bioacoustics, Berlin, Germany) was used for acoustical analysis. Spectrograms were created with a fast Fourier transform length of 512 points and an overlap of 75% (FlatTop window, 100% frame size), yielding a frequency resolution of 490 Hz and a time resolution of 0.5 ms. Calls were selected and classified manually from spectrograms by an individual masked to treatment conditions. Since few 22-kHz USVs (i.e. 20-30 kHz) were observed, we report only data for USVs of the 50-kHz type. Call rate was defined as the total number of 50-kHz calls per minute. All calls were categorized, as defined by Wright et al. (2010), into one of 14 subtypes or in approximately 1% of cases, into one of two categories. The two categories were: 'unclassifiable' (call was not loud enough or noise was present, preventing reliable subtyping) and 'miscellaneous' (call was visible but did not clearly fit one of the 14 subtypes).

Statistical analysis

Data were analyzed using commercial software (Systat v11, SPSS, Chicago, IL; GraphPad Prism4, La Jolla, CA). Use of parametric vs. nonparametric tests was guided by the distribution of the data (e.g. variance heterogeneity). Specific comparisons were performed using paired t-tests, Wilcoxon signed-rank tests or Sign tests. Differences between multiple vehicles were assessed by one-way ANOVA or Friedman's nonparametric analysis of variance. For all tests, a two-tailed p value less than 5 % was considered significant.

Experiment 1 Systemic administration of DAergic drugs

Experimental protocol

Initial amphetamine (AMPH) screen Before the main phase of testing, all rats (n=32) underwent an initial AMPH screen (Wright et al., 2010). This allowed us to remove the significant minority of rats that emit few 50-kHz vocalizations in response to systemic AMPH (Wright et al., 2010), as well as to increase the acute USV response to this drug in the remaining rats. Accordingly, rats were recorded for 20 min starting immediately after acute systemic injection of AMPH (1 mg/kg IP), on four occasions spaced 2 days apart. Ultrasonic vocalizations emitted during the 12^{th}, 14^{th} and 16^{th} minute on the 4^{th} day were counted. The 15 rats with the lowest call numbers were removed from the study, leaving a group size of 17.

Drug tests Testing resumed 7 days later. Each rat underwent two blocks of testing, which together comprised 19 test sessions spaced 2 days apart. In the first block (7 sessions), rats were tested with saline (twice) and AMPH (0.1, 0.3, 0.6, 1.6 and 4 mg/kg IP). The second block (12 sessions) comprised tests with a submaximal dose of AMPH (0.6 mg/kg) and a DA receptor agonist (A68930, quinpirole, or PD 128907), given either alone or in combination. All test drugs, doses, injection times and systemic routes of administration are shown in Table 1. Fully factorial within-subject designs were employed (i.e. each rat was tested under each drug condition). The drug and treatment conditions within each experiment were counterbalanced as nearly as subject numbers allowed. The session duration (30 min) and injection-test intervals were chosen to accommodate the behavioural time course of all drugs (Chapter 2: Scardochio and Clarke, 2013).

Drugs

The following drugs were used: the D1-like agonist A68930 hydrochloride, the D2/D3 agonist (-)-quinpirole hydrochloride (Sigma Aldrich, Oakville, ON), the D3 agonist (+)-PD 128907 hydrochloride (Tocris Bioscience, Minneapolis, MN) and D-amphetamine (Sigma-Aldrich, Poole, UK). All doses are expressed as salt and all drugs were dissolved in 0.9% sterile saline. The timing and pH of each control (vehicle) injection matched that of the respective drug. Drugs were administered in a volume of 1 ml/kg, either by the IP or subcutaneous (SC) route (see Table 1). All doses were chosen based on their effectiveness in multiple behavioural assays, as previously reviewed (Chapter 2: Scardochio and Clarke, 2013).

USV data analysis

Data analysis was restricted to selected time bins within each session (see Table 1 for details). For the first test block (AMPH alone), these were based on the known behavioural time course of AMPH-induced USVs (Wright et al., 2010; Wright and Scardochio unpublished data). For the second block (DAergic agonists/AMPH), time bins were evenly spaced across the session (see Table 1 for details).

Experiment 2 Fast-scan cyclic voltammetry

Electrochemical microsensor and reference electrode fabrication

Dopamine microsensors (i.e. working electrodes) and reference electrodes for chronic implantation were fabricated as previously described (Clark et al. 2010; Fortin et al. 2015). Briefly, a carbon fiber (grade 34-700; Goodfellow Corporation, Caraopolis, PA, USA) was

threaded through a fused silica shaft (Polymicro Technologies, Phoenix, AZ, USA) while submerged in 2-propanol. A short length (approximately 17 mm) of carbon fiber was left protruding and a small drop of two-component epoxy (Lepage Speed Set Epoxy^{TM}) was applied to the same end and allowed to dry for 3-5 hrs. Next, silver epoxy (MG Chemicals, Allied Electronics, Fort Worth, TX, USA) was applied to the other end of the silica shaft in order to secure electrical contact between the carbon fiber and a gold-plated beryllium-copper PCB socket (Newark Element 14 #23K7802, Chicago, IL, USA). The silver epoxy was left to dry overnight and then coated with two-component epoxy. The protruding length of carbon fiber was trimmed to 150-200 µm.

Reference electrodes were each made from a piece of silver wire (A-M systems, bare: 0.010", coated: 0.013"), cut to a length of approximately 1 cm and the plastic coating was removed. A small drop of silver epoxy was applied to the open end of a nickel-plated brass pin (Newark Element 14 #82K7794, Chicago, IL, USA) and the silver wire was inserted. Once dry, the silver epoxy was covered with two-component epoxy and the protruding silver wire was trimmed to approximately 3 mm. To create a silver/silver chloride surface, the protruding wire was soaked in a 10% sodium hypochlorite solution overnight.

Voltammetry surgery

Please note: the rats implanted with stimulating electrodes are the same four rats from Chapter 3. All surgical procedures followed aseptic technique. Rats were anesthetized with isoflurane (5% induction, 2-2.5% maintenance, AErrane, Baxter). The scalp was shaved and the animal was placed in a stereotaxic frame. Polyvinyl alcohol (1% w/v, HypoTears gel, Novartis) was applied to the eyes and a non-steroidal anti-inflammatory analgesic (carprofen, 5 mg/kg SC) and 0.9%

sterile saline (2 mL, SC) were administered. Baxedin^{TM} antiseptic (0.05% w/v chlorhexidine gluconate + 4% v/v isopropyl alcohol) and local anesthetic (50:50 v/v mixture of 2% lidocaine and 0.5% bupivicaine) were applied to the scalp, and then a midline incision was made. Skull holes were drilled and cleared of dura mater above the nucleus accumbens core (2.0 mm lateral and 1.7 mm rostral to bregma) and shell (0.9 mm lateral and 1.5 mm rostral to bregma). Two microsensors were implanted in each rat (core and shell, one in each hemisphere). In 4 of the 15 rats, a skull hole was drilled in order to position a stimulating electrode (Plastics One, MS303/2-A/SP) above the medial forebrain bundle (MFB; 1.3 mm lateral and 4.6 mm caudal to bregma). Four to five additional holes were drilled at convenient locations, to accommodate a reference electrode and 3-4 stainless steel anchor screws. The reference electrode was lowered 3-4 mm from the skull surface and one screw was secured in the skull; the reference electrode and screws were anchored with cranioplastic cement, leaving the stimulating electrode and microsensor holes exposed. For rats (n=11) without a stimulating electrode, the two microsensors were lowered (0.2 mm/min) into the target recording regions (7.4 mm ventral of the brain surface for core and shell of nucleus accumbens). For the remaining four rats receiving stimulating electrodes, the two microsensors were first attached to the voltammetric amplifier and then lowered (0.2 mm/min) into the target recording regions (7.4 mm ventral of the brain surface for core and shell of nucleus accumbens). In order to optimize the placement of the stimulating electrode, the voltammetric waveform was applied at 10 Hz and background electrical signal was monitored. Next, the stimulating electrode was lowered slowly (0.2 mm/min) to 7.2 mm below the dura mater, and electrical stimulation (24 biphasic pulses, 60 Hz, ± 120 μA and 2 ms per phase) was applied via an optically isolated, constant-current stimulator (A-M Systems, Sequim, Washington). If an evoked change in catecholamine concentration was not observed at the

microsensor, the stimulating electrode was lowered in 0.3 mm steps until electrically-evoked DA efflux was detected. The electrode was then lowered in 0.1 mm increments until DA release was maximal. This occurred when the stimulating electrode was between 8.4-8.7 mm ventral from the brain surface. If DA was not detected at this point, the stimulating electrode was lowered to 8.7 mm. Commonly, electrically-evoked DA is not detected during surgery (here, 3/8 electrodes) but becomes detectable once the electrodes have stabilized (e.g. 30 days later); this is thought at to be due, least in part, to heterogeneous microenvironments in the NAcc (Wightman et al. 2007). These microenvironments may support stimulated DA release but have a low *frequency* of transients; thus, these transients are less likely to be detected during the brief surgery recording. Finally, for all rats (n=15), cranioplastic cement was applied to the exposed skull to secure the microsensors, the 2-3 additional screws and for 4 rats, the stimulating electrode. For post-operative pain management, carprofen was administered every 24 hours for four days.

Voltammetric data acquisition

For details of hardware and software (see Fortin et al., 2015). Waveform generation and data acquisition were carried out by using two input/output cards (NI PCI-6052E an NI PCI-6711, National Instruments, Quebec, Canada) and software written in LabVIEW (National Instruments). Signals from chronically implanted microsensors were forwarded to the data acquisition system via a head-mounted voltammetric amplifier (current-to-voltage converter) and an electrical swivel (Crist Instrument Co Inc., Hagerstown, MD) mounted in the Faraday cage. The Faraday cage was a locally constructed metal cabinet that was grounded and insulated to exclude electrostatic and electromagnetic noise. The voltammetric amplifier comprised an operational amplifier with a feedback resistor (R_f 5 MΩ), in parallel with a 6 pF capacitor to

exclude high frequencies. To filter out operational amplifier noise, additional capacitors bridged each of the power sources (+15 V, −15 V) with ground. On each test day, to promote a stable background current, rats were connected to the head-mounted voltammetric amplifier and working electrodes were cycled with the voltammetric waveform at 60 Hz for 30 minutes and then at 10 Hz for 15 minutes (Moussy and Harrison 1994). It is not uncommon to observe a 200 mV shift in potential at reference electrodes within a few days (Heien et al., 2005). This shift can be detected by the position of the Faradaic peaks within the background current. When observed, this was corrected by applying a 200 mV offset to the waveform (Heien et al., 2005). Voltammetric recordings consisted of a series of fast voltage scans, repeated at 100 ms intervals (i.e. 10 Hz). Each scan lasted 8.5 ms and comprised an ascending and descending linear (400 V/s) sweep between -0.4 and +1.3 V, applied to the microsensors in relation to the Ag/AgCl reference electrode. The potential was held at −0.4 V versus the Ag/AgCl reference electrode between scans.

Experimental protocol

Overview Rats were tested one month after surgery to allow the electrodes to stabilize (Polikov et al. 2005; Kozai et al. 2014). Testing comprised four phases: (1) initial AMPH screen, (2) AMPH dose-response, (3) final AMPH screen (in a subset of rats) and (4) characterization of *in vivo* voltammetric signals. Six rats were removed from the study either because they did not survive the surgery or because they lost their cranioplastic implant during the month between surgery and testing (n=9 rats remaining).

Initial amphetamine screen Rats (n=9) underwent an initial AMPH screen which served to: 1) increase the acute response to AMPH and 2) to expose the rats to comparable conditions as in

Experiment 1. Rats were *not* connected to the head-mounted voltammetric amplifier to record DA transients during this time. Three 20-min sessions were given, spaced 2 days apart, each starting immediately after an acute systemic injection of AMPH (1 mg/kg IP, given in a volume of 1 mL/kg). Ultrasonic vocalizations emitted during the 12^{th}, 14^{th} and 16^{th} minutes on the 3^{rd} day were analyzed.

AMPH dose-response Testing resumed 5 days later. Rats were tested every other day, in seven test sessions. Each rat was tested under all conditions i.e. 5 doses of AMPH and saline (twice). Rats were connected to the head-mounted amplifier, and electrode cycling was performed to completion (see above). Starting immediately after injection, both phasic DA events and USVs were recorded for 30 min.

Final amphetamine screen Unexpectedly, rats challenged with AMPH called at a lower rate during the AMPH dose-response sessions than during the preceding AMPH screen. This raised the possibility that calling had been inhibited by the voltammetric headstage and tether, which were present only on test days. To assess this, a subset of rats (n=4) were subsequently retested with AMPH (1 mg/kg) in two sessions, spaced one day apart: 1) under the same conditions as the initial AMPH screen and 2) in the voltammetry test box and *not connected* to the headstage.

Characterization of in vivo voltammetric signals

These carbon fiber microsensors have been well validated for the detection of DA by FSCV (Wightman et al. 1988; Heien et al. 2003; Heien et al. 2004; Clark et al., 2010). However, our *in*

vivo voltammetry signals were further confirmed using four criteria: electrochemical, behavioural, pharmacological and anatomical, as follows.

Electrochemical validation (these data are the same as presented in Chapter 3) Electrical stimulation of the MFB can act as a positive control for the detection of DA using FSCV (Roitman et al. 2004). In the four rats implanted with stimulating electrodes, stimulation of the MFB was used to detect phasic DA release in the NAcc (24 equally spaced biphasic pulses, 2 ms/phase, 60 Hz, 120 μA).

Unexpected sucrose pellet Unexpected natural rewards increase phasic DA release in the nucleus accumbens (Day et al. 2007; Wassum et al. 2012; Park et al. 2012). Therefore, in the 5 rats that lacked stimulating electrodes, we monitored DA transients occurring in response to unexpected reward delivery, as follows. First, rats were habituated to sugar pellets (Dustless Precision Pellets®, 45mg sucrose, Bio-Serv) in their home cage for 3 consecutive days (~12 pellets/day). On test day, 5 unexpected sugar pellets were delivered via an externally-mounted rubber tube half-way through a 2-min voltammetric recording session.

Pharmacological validation Dopamine and noradrenaline are indistinguishable by FSCV alone (Heien et al., 2003). Therefore, once Experiment 2 was complete, electrochemical signals were further characterized; transients were recorded in all rats (n=8, one rat was removed due to electrode instability) following acute systemic drug injections. Voltammetric events were recorded for 30 min starting immediately after drug injection. In addition, electrically evoked DA was recorded at three time points: immediately before drug injection, and 15 and 35 min

after injection. The following drugs were tested: the D2/D3 receptor agonist (-)-quinpirole hydrochloride (Sigma Aldrich, Oakville, ON), the dopamine transporter blocker GBR12909 2HCl (NIMH Chemical Synthesis and Drug Supply Program), the D2/D3 antagonist raclopride, the α2-adrenoreceptor antagonist yohimbine (Tocris Bioscience, Minneapolis, MN), the norepinephrine transporter blocker desipramine hydrochloride (Sigma-RBI, St. Louis, MO) and D-amphetamine (Sigma-Aldrich, Poole, UK). Not all drugs were tested in each rat; see the Results section and Table S2 for details. All doses are expressed as the salt, and all drugs were administered by the IP route (see Table S1 for drug details). All drugs were dissolved in 0.9% sterile saline except for: GBR12909 in dimethyl sulfoxide (19% DMSO in saline) and yohimbine in distilled water. The timing and pH of each control (vehicle) injection matched that of the respective drug. Drugs were administered in a volume of 1 ml/kg except for GBR12909 (2 ml/kg).

Histological verification of recording sites Dopamine is the predominant electroactive neurotransmitter of the nucleus accumbens, and more specifically, in the lateral core (Garris et al. 1994) and rostral shell subregions (Park et al. 2010); thus, these were our targeted regions for microsensor implantation.

Once the pharmacological verification was complete (see above), the rats that retained intact head-implants (n=6) were deeply anesthetized with ketamine (100 mg/kg, IP) and xylazine (20 mg/kg, IP) and were transcardially perfused with saline and a 10% aqueous formalin solution (Sigma, St. Louis, MO). Brains were flash-frozen at -50ºC with 2-methylbutane (Acros Organics, New Jersey, USA), sectioned on a cryostat (25 μm coronal sections, -20 °C), mounted on microscope slides, and stained with Cresyl violet to better reveal anatomical structures. In 5 of

the 6 rats, an electrolytic lesion (+0.8 mA, 20s, direct current) was made before the perfusion step, to facilitate the histological identification of the recording site. This was done by applying current directly through each carbon fiber electrode. All electrode placements were histologically confirmed to be in the nucleus accumbens (Fig. S3).

Statistical analysis of FSCV data

Each recording electrode (two per rat) was treated as an independent unit. Even within a given animal, there is considerable variability between recording electrodes, likely reflecting: 1) the density of DA terminals at recording sites differ (Garris et al., 1994; Peters et al. 2000), 2) DA terminals within a given region differ in the extent to which they are autoinhibited (Moquin and Michael 2009) and 3) there exists spatial and temporal heterogeneity of DA transmission in the ventral striatum, where electrically-evoked responses differ in both amplitude and temporal profile (Wightman et al., 2007; Shu et al. 2013). We time-sampled the voltammetric and USV recordings for analysis, and the following minutes were selected based on the known behavioural time course of AMPH-induced USVs: 13, 16, 19, 22 and 25. Electrochemical data were analyzed using software written in LabVIEW 7.1 (National Instruments). For every recording session, the chemical signature (cyclic voltammogram) of drug-evoked phasic DA release was statistically compared to several template cyclic voltammograms of DA. These templates (i.e. training set) were obtained from electrically evoked DA release after stimulation of the MFB (Clark et al., 2010). This comparison was done by principal component analysis (PCA) (Keithley et al. 2009). Four principal components were retained, based on Malinowski's F test (Einax 2006), accounting for >99.5% of the variance. The quality of the fit was evaluated by comparing the predicted and observed data. The residual sums of squares value (i.e. Qt) was compared to a

critical value (Qα). This threshold for significance is set by the amount of noise in the recorded data based on the amount of error contained in the discarded principal components. Namely, if the Qt values exceed the Qα, then the recorded signal contains noise that exceeds that which is anticipated from the remaining principal components. Qt must then be greater than 1.645 (90% confidence interval) to define a transient event, and this criterion must be achieved for at least two consecutive scans (Cheer et al. 2004). Verification of our electrically-evoked and sugar pellet-evoked DA signals was performed using the 'CV match' algorithm (Wightman et al., 1988; Heien et al., 2004; Clark et al., 2010) written in CV Analysis (LabVIEW 7.1, National Instruments). Briefly, the resultant cyclic voltammogram was compared with a template voltammogram obtained from an electrically-evoked (24 equally spaced biphasic pulses, 2 ms/phase, 60 Hz, 120 μA) *in vivo* recording, and a correlation coefficient was obtained. The phasic event was determined to be dopaminergic if the correlation coefficient was ≥0.75 and transients were required to meet this criterion for at least two consecutive scans (Heien et al., 2003; Cheer et al., 2004).

Results

Experiment 1 Systemic administration of DAergic drugs

Initial amphetamine screen

During this initial screen, the median 50-kHz call rate was 17 calls per min, with considerable inter-rat variability (interquartile range (IQR) 3-50, n=32 rats). After exclusion of low callers (see "Methods"), the median call rate was 46 calls per min (IQR 27-65, n=17 rats). During this screen, and in subsequent testing, 22-kHz calls were seldom observed; hence, they are not reported here.

Amphetamine dose-response

One rat emitted no calls in any condition and was therefore eliminated from the study, leaving 16 rats for subsequent analysis. Call rates during the two control (saline) tests were averaged for each rat, as they did not differ significantly (Wilcoxon's signed-rank test, NS). AMPH significantly increased calling at two middle doses: 0.6 and 1.6 mg/kg (Wilcoxon: $p<0.01$ and $p<0.001$, respectively; Fig. 1).

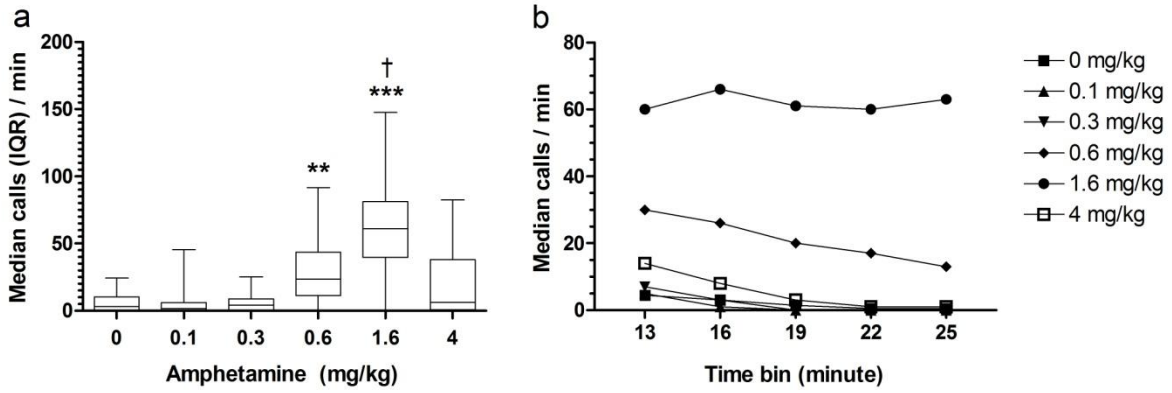

Fig.1 Experiment 1: Dose-dependent (a) and time-dependent (b) effects of amphetamine (0, 0.1, 0.3, 0.6, 1.6 and 4 mg/kg IP) on 50-kHz call-rate (n=16 in both panels). Panel **a** is a box plot showing median + IQR with range (min and max) represented by whiskers. Panel **b** shows median calls per 1-min time bin within the 30-min session. ** $p<0.01$, *** $p<0.001$ vs. saline (0 mg/kg) ; † $p<0.01$ vs. dose 4 mg/kg

Receptor subtype-selective dopamine agonists

The counterbalanced block of DA agonist testing included three tests with saline and three tests with AMPH alone (0.64 mg/kg). Since the call rates were statistically indistinguishable within each of these two conditions, the saline and AMPH data were separately averaged for each rat.

As expected, call rates were low after saline administration and were increased by AMPH when given alone (Fig. 2b, Wilcoxon: $p<0.001$).

When the three DA agonists were given alone, only quinpirole (D2/D3-selective) significantly reduced the 50-kHz call rate (Wilcoxon: $p<0.01$; Fig. 2a); this drug almost abolished calling. Agonist effects on AMPH-induced calling were assessed by comparing AMPH-saline difference scores, i.e. with vs. without agonist pretreatment (Fig. 2b). Both the D1-like and the D2/D3-selective agonists decreased AMPH-induced 50-kHz call emission (Wilcoxon, $p<0.002$).

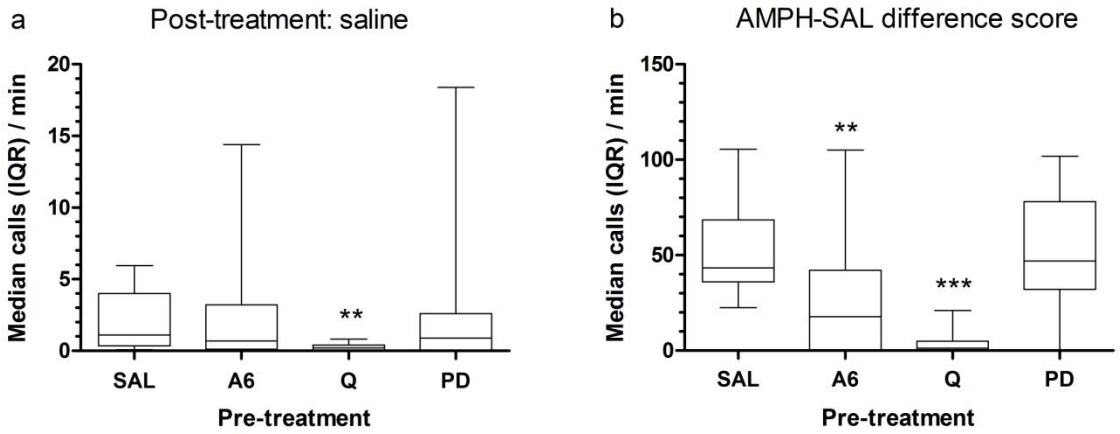

Fig. 2 Experiment 2: Effects of dopamine receptor subtype-selective agonists on (a) spontaneous 50-kHz calling and (b) amphetamine-induced 50-kHz calling (i.e. with the corresponding saline call rate subtracted). **a** The D1 agonist A68930 (A6; 0.25 mg/kg SC), D2/D3 agonist quinpirole (Q; 0.1 mg/kg IP), D3 agonist PD 128907 (PD; 0.01 mg/kg, SC) or saline (SAL) was administered before a saline injection. **b** A68930, quinpirole and PD 128907 administered before an amphetamine (0.64 mg/kg IP) injection. Each rat (n=16) was tested under all conditions. Calls/min were calculated from selected time bins (minutes 13, 16, 19, 22 and 25 of the 30-min

session) and are represented as median + IQR with range (min and max) described by whiskers. **p<0.01, ***p<0.001 vs. SAL, Wilcoxon's tests

Experiment 2 Fast-scan cyclic voltammetry

Characterization of the voltammetric signal

Electrically-evoked release Dopamine was detected at all but one recording site, as revealed by background-subtracted cyclic voltammograms (Fig. S1), following electrical stimulation of cell bodies in the MFB.

Unexpected sucrose pellet Sucrose delivery reliably evoked phasic DA release at 4 of the 6 electrodes (Fig. S2). Consistent with previous reports (Robinson and Wightman, 2007), multiple recording sessions (~3) were required to observe this increase in phasic DA in response to the unexpected pellet delivery.

Pharmacological verification All electrodes tested (n=5) displayed the expected pharmacological responses. Thus, electrically-evoked peak DA current significantly increased following an acute administration of the indirect DA/NA agonist amphetamine (paired t-test $p<0.05$), the D2 antagonist raclopride (paired t-test, $p<0.05$) and the DAT blocker GBR12909 (Fig. S2, paired t-test, $p<0.02$). The three other pharmacological conditions could not be assessed statistically; this is because two rats died prematurely, leaving only one rat tested per remaining condition. The first rat died within 24 hours of receiving DMI/YO, and so this drug combination was not tested in the other subjects. The second rat succumbed to anesthesia while the cranioplastic implant was being repaired. See Table S1 for details of completed drug conditions for each rat.

Histology Most (10/12) electrode placements were histologically located in the NAcc (Fig. S3). The remaining electrodes (#2 for rats 4 and 10) could not be located since no corresponding electrolytic lesion was observed.

Call rates: initial AMPH screen, AMPH dose-response block and AMPH post-screens

During the initial AMPH (1 mg/kg IP) screen, the median call rate was 58 calls per min, (IQR 53-83) and all rats (n=9) were retained. During the AMPH dose-response block, the two control (saline) tests did not significantly differ with respect to 50-kHz call rates (Wilcoxon's signed-rank test, NS), and these data were averaged for each rat. AMPH significantly increased calling at the three highest doses tested: 0.6, 1.6 and 4 mg/kg (Wilcoxon: $p<0.05$-0.02; Fig. 3, n=9). The median (IQR) calls per min for saline and AMPH doses 0.1, 0.3, 0.6, 1.6 and 4 mg/kg were as follows: 3 (0.8-9.6) , 1 (0-3), 1 (0-4), 5 (2-7), 33 (22-41) and 4 (0-17).

Call rates during the AMPH dose-response block were lower than during the initial screen. This finding suggested that during voltammetric recording, call emission might have been inhibited by tethering to the electrical swivel. This notion was supported by a follow-up experiment in which a subset (n=4) of animals was retested with AMPH (1 mg/kg IP), while untethered: once in the test box used in the initial screen, and subsequently in the voltammetric test box. Call rates (median, IQR, per min) were 62 (52-73) and 68 (64-71), respectively; call rates were generally higher than for the same rats during the AMPH dose-response tests.

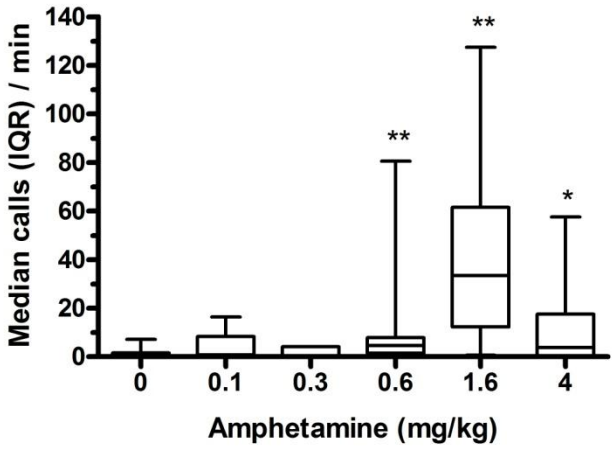

Fig. 3 Dose-dependent effect of amphetamine on 50-kHz call rate. Amphetamine increased 50-kHz vocalizations at the three highest doses tested. *p<0.05; **p<0.02 vs. zero dose (Wilcoxon's tests, n=9 rats). Call rate is median + IQR with range (min and max) represented by whiskers.

Effects of amphetamine on spontaneous phasic DA events and relation to USV emission

For reasons described above, data from 8 electrodes were excluded, leaving 10 electrodes in the final analysis (see 'Statistical analysis of FSCV data' and 'Characterization of the voltammetric signal'). Based on an initial analysis (Wilcoxon tests), each phasic DA parameter (number of DA transients, transient duration and peak DA current) was individually averaged across the two saline sessions for each rat. AMPH increased the mean peak DA current, with a significant effect at all doses except the lowest (paired t-tests with, p<0.02-0.05, Fig. 4b); in contrast, the frequency and duration of DA transients appeared unaffected (paired t-tests with HB correction, NS, Fig. 4a and c).

Of particular note, AMPH increased the rate of 50-kHz call emission (see above) without detectably increasing the occurrence of transient DA release events. Overall, 50-kHz calls outnumbered DA transients by a factor of 4-10 (depending on dose), and there was very little co-

occurrence between the two types of events. Thus, for all treatment conditions (saline and five amphetamine doses), the majority (> 95%) of calls occurred in the absence of a phasic DA event (within 0.2 s before or after call onset; Table 2). When the criterion for call-occurrence was expanded to 1 s before or after call onset, the % of calls occurring in the absence of transients remained unchanged. The median percentage of calls co-occurring with transients, for saline and AMPH doses 0.1, 0.3, 0.6, 1.6 and 4 mg/kg were as follows: 0 (0-0), 0 (0-0.8), 0 (0-0), 0 (0-1.1), 3.7 (1.9-5.6) and 3.9 (0-20).

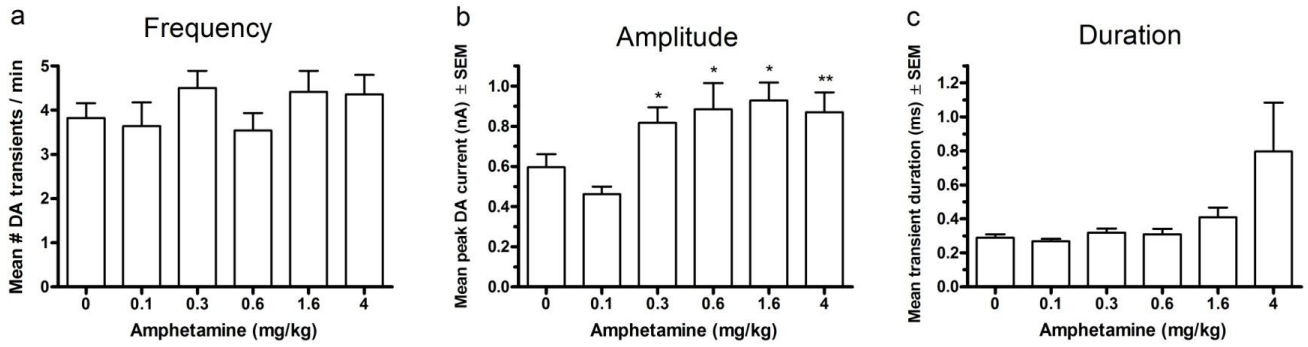

Fig. 4 Effects of amphetamine on frequency, amplitude and duration of phasic DA events (n=10 electrodes). AMPH did not significantly increase the mean frequency (panel **a**: repeated-measures ANOVA, NS) or mean duration (panel **c**: Wilcoxon tests, NS) of these events. However, AMPH significantly increased the mean amplitude of phasic DA at all doses tested except the lowest. *p<0.05; **p<0.02 vs. zero dose, paired t-tests).

Table 1 Summary of drug conditions for each experiment and phase

Exp	Pre-treatment					Post-treatment					Analysis	
	Drug		Dose (mg/kg)	Route	Time before testing (min)	Drug		Dose (mg/kg)	Route	Time before testing (min)	Session length (min)	Minutes sampled
1	----	----	----	----	----	DA/NA indirect agonist	D-amphetamine	0, 0.1, 0.256, 0.64, 1.6, 4	IP	administered immediately before testing	30	13, 16, 19, 22, 25
2[a]	D1-like agonist	A68930	0, 0.25	SC	20			0, 0.64				5, 10, 15, 20, 25
	D2/D3 agonist	Quinpirole	0, 0.1	IP	5							
	D3 agonist	PD 128907	0, 0.01	SC	10							

[a]Each rat was also tested with amphetamine (1 mg/kg IP, positive control) within the counterbalanced design.

Discussion

Relationship between amphetamine dose and USV response

Consistent with previous studies (Wright et al., 2010a; Wohr et al., 2015), AMPH maximally increased 50-kHz call rates at doses of 1-2 mg/kg. By extending the AMPH dose range, we have revealed an inverted-U relationship, with the highest AMPH dose (4 mg/kg) producing little or no increase in call rate. A comparable profile occurs for the locomotor stimulant effect of AMPH (Sahakian et al. 1975; Finn et al. 1990), although the two behavioural effects are pharmacologically dissociable (for review see: Knutson et al., 2002). As AMPH doses are raised, locomotor stimulation is eventually curtailed through the emergence of focal stereotypy (Segal 1975; Joyce and Iversen 1984). However, it is unclear whether the stereotyped behaviour observed at our highest dose would also have inhibited the emission of 50-kHz calls, particularly since high doses of AMPH tend to promote sniffing behaviour (Sharp et al. 1987), and 50-kHz calls are synchronized with exhalation (Sirotin et al. 2014).

D1-like and D2/D3 agonists inhibit AMPH-induced 50-kHz vocalization

In the present study, we confirmed that the D2/D3 agonist quinpirole decreases spontaneously-emitted 50-kHz vocalizations (Chapter 2: Scardochio and Clarke, 2013). Although we were unable to confirm a depressant effect of D1-like and D3 agonists (Chapter 2: Scardochio and Clarke, 2013), this was likely due to a floor effect, as call rates under saline were low. For testing with DA receptor ligands, we selected a low dose of AMPH from the ascending limb of the dose-response curve. AMPH-induced calling was inhibited by DAergic antagonists, as previously reported (Wright et al., 2013; Wohr et al., 2015). An inhibitory effect was also seen with D1-like and D2/D3 receptor *agonists*. This finding, together with the earlier failure of DA blockade to

evoke calling (Wright et al., 2012), supports a role for phasic rather than tonic DA transmission in 50-kHz call emission. This is because AMPH is reported to increase phasic DA release (Daberkow et al., 2013; Covey et al., 2013), and the postsynaptic impact of DA would be blunted by direct-acting DA receptor agonists as well as antagonists.

Acute systemic amphetamine increases the amplitude of phasic DA release

To our knowledge, this is the first report of the effects of low- and intermediate-doses of AMPH on phasic DA release in the NAcc, in freely moving animals. In Experiment 2, all doses of AMPH except the lowest increased the size of phasic DA events, without detectably altering their frequency or duration. AMPH is widely thought to act by depleting vesicular DA stores and by promoting non-exocytotic DA efflux via reversal of the DA transporter (Sulzer 2011), actions that would have resulted in longer, but not larger, DA transients. However, AMPH can also promote vesicular DA release via burst firing of DA neurons, as recently described in anesthetized animals (Ramsson et al. 2011a; Ramsson et al. 2011b). A recent study in freely-moving rats provides evidence for multiple mechanisms (Daberkow et al., 2013); specifically, AMPH (1 and 10 mg/kg) increased the duration and frequency of spontaneous DA transients, but the higher dose also increased release *amplitude* in a few animals. These effects were studied only in the dorsal striatum, but the enhancement of release amplitude would likely be greater in the NAcc, based on measurements of electrically-evoked phasic DA release in anesthetized animals (Covey et al., 2013). Taken together, then, the increased amplitude of phasic DA events that we observed may have been caused by an increase in vesicular DA release.

Phasic DA release in the nucleus accumbens is neither necessary nor sufficient for AMPH-induced USVs

Experiment 2 showed that while amphetamine increased both 50-kHz call rates and the amplitude of spontaneous phasic DA release events, these two effects could be dissociated in two ways. First, an inverted-U dose-response relation was only seen in relation to 50-kHz call emission. Second, 50-kHz calls and DA transients rarely coincided. In particular, the great majority (>95%) of calls occurred in the absence of detectable DA transients, and most DA transients were not accompanied by calls. It is unlikely that a significant number of DA transients went undetected, since our FSCV method is associated with a detection limit of approx. 10 nM (Robinson and Wightman 2007), i.e. considerably below the average peak amplitude of *spontaneous* DA events in the NAcc (approx. 50 nM) (Robinson and Wightman, 2007). Taken together, therefore, our findings indicate that phasic DA release, as monitored at our NAcc recording sites, was not sufficient to increase 50-kHz calls, nor was it necessary. However, we cannot exclude the possibility that AMPH promotes 50-kHz call emission by enhancing phasic DA release in a terminal field other than NAcc core. The NAcc comprises two major subregions (core and shell), and it is important to note that our voltammetric recordings were mostly restricted to the core subregion (6/10 electrodes) or the core/shell shore (2/10). As a recording site, the core has one major advantage compared to the NAcc shell: voltammetric signals in the core are not appreciably contaminated by extracellular NA (Garris et al., 1994). Whether AMPH differentially affects phasic DA release in core vs. shell appears unknown, although differences in phasic DA release have been noted between other terminal fields (Covey et al., 2013).

Methodological aspect

Rats called significantly less during the dose-response (voltammetry) test block than during the preceding or subsequent AMPH screens; call subtype profiles, in contrast, appeared unaffected. Thus, it appears likely that the voltammetric procedure, and tethering in particular, was responsible for the general inhibition of 50-kHz call rate. However, it seems implausible that such an effect would obscure a relationship between call emission and phasic DA events.

Conclusion

Tonic agonist-induced activation of D1- or D2-like dopamine receptors inhibited AMPH-induced 50-kHz calling, suggesting that 50-kHz calls are driven by phasic DA release events. However, most AMPH-induced calls occurred in the absence of DA transients in the NAcc core, indicating that 50-kHz call emission does not depend critically on phasic DA release in this DA terminal field. Whether 50-kHz call production depends on phasic DA release events in other brain regions requires further investigation. Lastly, the novel finding that moderate doses of AMPH can increase the magnitude of phasic DA events in freely-moving animals may help to shed light on the drug's myriad behavioural effects.

CHAPTER 5: General Discussion

Tina Scardochio

Summary

This thesis examined the role of DA in the production of 50-kHz USVs. Our initial approach was to use systemic drug injections to selectively activate or inhibit DA receptor subtypes (Chapter 2). Both classes of drugs inhibited 50-kHz calling. From this, we concluded that increased DAergic transmission is not sufficient to increase 50-kHz calls, whereas DAergic transmission is necessary for USV production.

To reconcile the shared effects of DA agonists and antagonists on 50-kHz call rate, we proposed that, more specifically, phasic DA drives 50-kHz call emission. This hypothesis was tested in Chapter 3, in two ways. First, we confirmed that 50-kHz calls could be induced by electrical stimulation of the MFB; using FSCV in freely moving animals, we found that these electrically-evoked calls were time-locked to phasic DA release in the NAcc. Second, optogenetic stimulation of VTA DAergic neurons was associated with a high 50-kHz call rate, comparable to that observed following other positive reinforcers; however, call rates decreased quickly over time, despite prolonged optogenetic stimulation. To explain this discrepancy, we entertained and then rejected alternative explanations (channelrhodopsin-2 desensitization and habit formation of lever-pressing) with a follow-up experiment. We also proposed additional explanations including DA receptor desensitization and glutamatergic contributions. From the findings in Chapter 3, we concluded that phasic DA release in the NAcc was not sufficient to induce 50-kHz calls.

In the final experimental section (Chapter 4), we again investigated a possible role of phasic DA release in USV emission, but now focussing on *amphetamine*-induced 50-kHz calls.

Amphetamine had been shown to increase 50-kHz calls and also phasic DA release in the NAcc. Here, we found that tonic activation of DA receptors by DA receptor subtype-selective agonists decreased AMPH-induced calls. While amphetamine increased the amplitude of DA transients in the NAcc as well as the 50-kHz call rate, the two were not correlated; notably, most calls occurred in the absence of detectable phasic DA release in the NAcc, suggesting that phasic DA is not necessary for the emission of 50-kHz calls.

Taken together, the results presented in this thesis are consistent with a role of DAergic signaling in the emission of 50-kHz USVs. However, phasic DA release in the NAcc appears neither sufficient nor necessary for call emission. The emission of spontaneous and AMPH-induced 50-kHz calls likely requires the concerted actions of multiple neurotransmitter systems in several brain regions.

The potential involvement of other brain regions and neurotransmitters in the emission of 50-kHz calls

Pharmacological experiments in Chapters 2 and 4, as well as the electrical stimulation studies in Chapter 3, support a role for phasic DA release in the emission of 50-kHz calls. In contrast, the optogenetic stimulation (Chapter 3) and phasic DA recordings (Chapter 4) results suggest that phasic DA in the NAcc is neither sufficient nor necessary for the production of 50-kHz calls. However, it remains possible that phasic DA is necessary or sufficient in: 1) other parts of the NAcc or 2) other brain regions. It is also possible that phasic DA release *with* 3) release of other neurotransmitters is required. I will next discuss evidence to support each of these possibilities.

1) In Chapters 3 and 4, phasic DA release was recorded from the NAcc, a region shown to play a role in the production of 50-kHz calls (Burgdorf et al. 2001; Thompson et al. 2006; Burgdorf et al. 2007; Brudzynski et al. 2012). The NAcc is heterogeneous and can be divided based on: the core and shell anatomical divide (Deutch and Cameron 1992) and shell subregions (Heimer et al. 1997; Shin et al. 2008). These NAcc subregions are distinct in terms of distribution patterns of neuroactive substances (Zahm 1999), afferent and efferent projections (Meredith et al. 1992; Wright and Groenewegen 1995) and functional aspects (Heidbreder and Feldon 1998; Ikemoto 2002; Sellings and Clarke 2003; Meredith et al. 2008; Fabbricatore et al. 2009; Fabbricatore et al. 2010; Reed et al. 2015). Phasic DA release in the NAcc core and shell subregions is differentially regulated by various stimuli including food reward (Brown et al. 2011) and drug rewards (Owesson-White et al. 2009) and aversive footshock (Badrinarayan et al. 2012). Microinjection of amphetamine into the NAcc shell rather than the core, has been shown to increase 50-kHz call rate (Burgdorf et al., 2001; Thompson et al., 2006; Brudzynski et al., 2012), whereas in this thesis, the majority of phasic DA recordings were in the NAcc core. It remains possible that phasic DA release in the NAcc shell or shell subregions is also involved in the production of 50-kHz calls, either similarly or differently than core phasic DA. Alternatively, NA release may explain the call rate-enhancing effect of intra-NAcc AMPH previously reported given that the shell contains more NA than the core (Park et al. 2010; Park et al. 2011) and AMPH is an indirect DA/NAergic agonist.

2) We cannot exclude the involvement of phasic DA release in other brain regions in the production of 50-kHz calls. There is a large functional heterogeneity of VTA outputs (Walsh and Han 2014). Burgdorf et al. (Burgdorf et al. 2008) have reported a potential role for several other

brain regions in the emission of 50-kHz calls, including the prefrontal cortex and lateral hypothalamus which both receive projections from the VTA (Swanson 1982). However, in the latter report, data from several brain regions were pooled, preventing analysis of the relative contributions for each region. Phasic DA release would also occur in many terminal regions following electrical stimulation of the MFB (Chapter 3 and 4) or optogenetic stimulation of the VTA (Chapter 3).

3) The focus of my thesis was the role of *dopamine* in the emission of 50-kHz calls; however, several additional neurotransmitters have been shown to play a role in the emission of USVs, as follows. First, antagonism of glutamatergic NMDA receptors has been reported to decrease spontaneous, AMPH- and tickling-induced 50-kHz vocalizations (Panksepp and Burgdorf 2000; Costa et al. 2015). Conversely, intracerebral injections of glutamate induce spontaneous 50-kHz calls (Fu and Brudzynski 1994; Wintink and Brudzynski 2001). Second, the muscarinic/nicotinic ACh agonist carbachol increased spontaneous 50-kHz calling after intra-NAcc injections (Fendt et al. 2006). Third, microinjections of a μ-opioid agonist directly into the VTA promoted 50-kHz calling and also produced a conditioned place preference (Burgdorf et al., 2007). Conversely, opioid antagonists (selective for μ- or κ-selective receptors) inhibited spontaneous calling (Hamed et al. 2015) and calling in anticipation of food (Buck et al. 2014). Fourth, inhibition of noradrenergic signaling decreased spontaneous and AMPH-induced 50-kHz call rate (Blumberg et al. 2005; Wright et al. 2012). Fifth, systemic administration of a 5-HT(1A) receptor agonist increased 50-kHz calls (Sadananda et al. 2012) and 5-HT(2C) antagonists blocked AMPH-induced 50-kHz calls (Wohr et al. 2015). Lastly, inhibition of endocannabinoid hydrolysis has been linked to increases in play-associated 50-kHz call emission (Manduca et al. 2014). Of

interest, all of these neurotransmitters either directly or indirectly affect the mesolimbic DA system that was investigated in this thesis (see next section).

Interactions between dopamine and other neurotransmitters

All the neurotransmitters that have been shown to modulate 50-kHz emission - acetylcholine, cannabinoids, glutamate, opioids, noradrenaline and serotonin - can modulate mesolimbic DA release and transmission (Cheer et al. 2004; Mark et al. 2011; Mitrano et al. 2012; Picciotto et al. 2012; Wenzel and Cheer 2014; Margolis et al. 2014; Chenu et al. 2014; Latagliata et al. 2014; Eddine et al. 2015). Electrical stimulation of the MFB (Chapter 3) is neurochemically non-specific; given that many neurotransmitter systems course along its path, DA fibers are not the only ones activated (Ikemoto 2010). Optogenetic stimulation of VTA DAergic neurons afforded us a higher degree of selectivity: only DAergic neurons whose cell bodies were found in the VTA were activated during optical stimulation. Despite this regional selectivity, the population of neurons intrinsic to the VTA collectively include at least two additional neurochemical phenotypes aside from DA: GABA and glutamate. It has recently been shown that both GABA (Tritsch et al. 2012) and glutamate (glut) (Stuber et al. 2010) can be co-released from DAergic terminals. However, in Chapter 3, we did not record phasic DA release during optogenetic stimulation; therefore, we cannot exclude the possible involvement of DA-glut or DA-GABA co-release on 50-kHz call emission or lever-pressing behaviour. Moreover, the separation of the anterior and posterior VTA is paralleled by behavioural responses to drugs of abuse (Sanchez-Catalan et al. 2014). However, our viral infections would be expected to affect the entirety of the VTA, and even portions of the adjoining substantia nigra, based on previous

immunohistochemistry results from the Shizgal lab; thus potential differences associated with the antero-posterior gradients would not likely be observed.

Briefly, using real-time recordings in freely moving animals, we investigated the effects of DAergic signaling on 50-kHz call production. However, given the considerable cellular, molecular and regional heterogeneity present in the VTA, these tools are not without limitations (see next section). Future technological advancements should impart additional specificity and selectivity to better interrogate the underlying circuits involved in the production of USVs.

Tools with which to interrogate the circuits involved in 50-kHz call emission

In this thesis, FSCV and optogenetics were used to investigate a single transmitter pathway; however, these techniques have a wider applicability. In this next section, I will discuss four additional applications for FSCV and optogenetics that will potentially address the questions arising from the results presented in the thesis chapters.

1) In order to study the differential involvement of DAergic signaling in the core vs. shell of the NAcc in the emission of 50-kHz calls, FSCV and optogenetics could be used in conjunction with recombinant adeno-associated viral vectors (rAAVs). More specifically, rAAVs can be injected into either the core or shell of the NAcc and retrogradely traced back to the VTA, resulting in projection-specific expression of channelrhodopsin-containing DAergic neurons (Masamizu et al. 2011; Rothermel et al. 2013). This would allow us to specifically investigate the potential roles of phasic DA in the core vs. shell, in the emission of 50-kHz calls.

2) We used FSCV to record phasic release of DA; however, FSCV can also be used to measure other neurotransmitters that are proposed to play a role in the emission of 50-kHz calls. Until recently, voltammetry techniques such as FSCV could only detect molecules that are intrinsically electroactive (e.g. DA, NA, 5-HT). However, recent advances, particularly in electrode design, have helped to overcome this limitation in some cases. For example, the recording electrode can be modified with an enzyme that is selective for the molecule of interest. Such enzyme-based sensors have been created for several non-electroactive neurotransmitters, including glutamate (Oldenziel and Westerink 2005), acetylcholine (Sarter et al. 2009) and its precursor choline (Parikh et al. 2004). Combining optogenetics with FSCV using these enzyme-based sensors would help us investigate the potential contributions of glutamate (following co-release with DA) to our findings in Chapter 3.

3) In Chapter 3, optogenetic tools were used to selectively activate VTA DAergic neurons. Transgenic rat lines were used in which the Cre recombinase was expressed under the regulatory control of the promoter for tyrosine hydroxylase, and channelrhodopsin 2 was injected into the VTA using a viral vector. This technique allowed us to selectively activate an expected 60-70% of DAergic neurons (Oldenziel and Westerink, 2005; Witten et al. 2011). However, improved viral transfection methods using magnetoparticles now allow for up to 97% transfection efficiency (Soto-Sanchez et al. 2015). This technical refinement would likely have produced a greater increase in phasic DA release in downstream regions which may have affected the emission of 50-kHz vocalizations. One possibility is that a threshold of phasic DA release must be achieved in order to produce 50-kHz calls. Activation of a larger number of DAergic neurons

may compensate for the potential decrease of phasic DA release later in the session (due to for example, DA depletion, as discussed in Chapter 3).

4) In Chapter 3, we used optogenetic stimulation to excite VTA DAergic neurons. It would be of interest to see if selective *inhibition* of those same neurons would inhibit 50-kHz call production. Neuronal inhibition can be achieved by using inhibitory opsins such as archaerhodopsins (Chow et al. 2010) or halorhodopsins (Boyden et al. 2005); different wavelengths of light can be used to selectively activate inhibitory and excitatory opsins transfected in a given brain region (Han and Boyden 2007). While optical suppression of *cocaine*-evoked phasic DA release has already been shown in urethane-anesthetized rats (Oldenziel and Westerink, 2005; McCutcheon et al. 2014), it would also be of interest to investigate whether inhibition of VTA DAergic neurons is sufficient to block AMPH-induced phasic DA release and 50-kHz calls.

Analyzing potential complexities in rat 'language'

In the literature, rat USVs are often broadly divided based on their two main emitted frequencies and their association with negative and positive affect: 22-kHz and 50-kHz calls, respectively. In all previous chapters, more than 98% of all calls in all experiments were of the 50-kHz call subtype, and 22-kHz calls were not analyzed or reported. However, when 22-kHz calls did occur, they were intermingled with 50-kHz calls (unpublished) suggesting that 50-kHz and 22-kHz calls are not always dissociable based on affect. Recently, the behavioural correlates of the 50-kHz call type heterogeneity are starting to be explored. For example, the 'trill' call subtype of 50-kHz calls is predominantly emitted during copulation (Snoeren and Agmo 2014) and play behaviour (Burgdorf et al., 2008). In addition to the 14 call subtypes so far identified, call parameters such

as harmonics, frequency modulation and amplitude may perhaps also be used as markers for other behaviours such as novelty-, reward- and sensation-seeking (Yuki and Okanoya 2014; Garcia et al. 2015)

In addition to the 50-kHz call type heterogeneity, there is another facet of 50-kHz vocalizations that has yet to be explored: call sequencing and syntax. 'Language' has been extensively studied in other animal species such as the zebra finch, and has been used to study aspects of autism (Kleiman et al. 2015), addiction (Lovell et al. 2011) and communication (Matheson et al. 2015), among other disorders. Thus, it appears likely that we would gain a wealth of knowledge from improved rat models following the comprehensive analysis of rat vocalization sequences. We recently began this type of call analysis (unpublished, in collaboration with Dr. Jon Sakata, Biology department, McGill University) and have so far discovered that: 1) call sequences are more structured than would be expected by chance, both under baseline (non-drug) conditions and following acute amphetamine administration, 2) this sequence structure reflects, at least in part, the tendency to repeat a given call subtype, and 3) amphetamine does not increase such call-repeats above what is already observed under saline conditions. Currently we are investigating call transitions, the likelihood of repeating specific call subtypes, the individual contributions of call subtypes to entropy, the existence of call bouts, the effects of call intervals, and lastly, how various doses of amphetamine may affect each of these parameters.

Given our current knowledge of USVs, these vocalizations have been incorporated into several research models including autism (Zhang-James et al. 2014), addiction (Mahler et al. 2013), Parkinson disease (Grant et al. 2015) and arthritic pain (Calvino et al. 1996). These calls have

also been proposed as a model to screen drugs for antidepressant and anxiolytic properties (De et al. 1993; Pereira et al. 2014). Translational studies have even compared spectral and temporal features of human infant cries during cocaine withdrawal to rat pup USVs following prenatal exposure to cocaine (Zeskind et al. 2014). However, for translational research of this kind, assay selectively is critical in order to minimize the occurrence of false positives. In this respect, some current assays require further development. For example, many drugs that decrease 50-kHz calls (e.g. DA agonists) could be incorrectly assumed to be viable treatments for anxiety. Similarly, assuming that all 14 subtypes of 50-kHz calls reflect affect, other potentially important behavioural distinctions may be overlooked (e.g. wanting vs. liking; Berridge et al. 2009).

Concluding remarks

Adult rat 50-kHz vocalizations are frequently used an index of positive affect. However, previous work and the results presented here have shown that these calls are not always linked to situations that would otherwise be associated with positive affect (e.g. self-administration of reinforcing optical stimulation). Therefore, we must tread lightly when making generalized statements about their behavioural role or meaning of 50-kHz calls. Our hope is to combine the neurochemical data (present in this thesis and elsewhere in the literature) with the analysis of temporal and acoustic structure as well as behavioural outcomes, to eventually decode the functional significance of rat USVs; the culmination of this work should lead to better rat models and ultimately, a better understanding of disease states. Once we describe the neurocircuitry underlying USVs, changes in call structure (acoustic, sequence, temporal, etc.) in animal models of disease could point to dysfunctions in the neurocircuitry of the pathological states themselves. Similarly, once we describe the behavioural basis of USVs in more detail, changes in USV call

rates or parameters in rat models of disease could be relayed back to these known behavioural phenotypes of 50-kHz calls or call subtypes.

Reference List

Acquas E, Di Chiara G (1994) D1 receptor blockade stereospecifically impairs the acquisition of drug-conditioned place preference and place aversion. Behav Pharmacol 5: 555-569

Ahrens AM, Ma ST, Maier EY, Duvauchelle CL, Schallert T (2009) Repeated intravenous amphetamine exposure: rapid and persistent sensitization of 50-kHz ultrasonic trill calls in rats. Behav Brain Res 197: 205-209

Ahrens AM, Nobile CW, Page LE, Maier EY, Duvauchelle CL, Schallert T (2013) Individual differences in the conditioned and unconditioned rat 50-kHz ultrasonic vocalizations elicited by repeated amphetamine exposure. Psychopharmacology (Berl) 229: 687-700

Allen TA, Furtak SC, Brown TH (2007) Single-unit responses to 22 kHz ultrasonic vocalizations in rat perirhinal cortex. Behav Brain Res 182: 327-336

Al-Naser HA, Cooper SJ (1994) A-68930, a novel, potent dopamine D1 receptor agonist: a microstructural analysis of its effects on feeding and other behaviour in the rat. Behav Pharmacol 5: 210-218

Andersen PH, Jansen JA (1990) Dopamine receptor agonists: selectivity and dopamine D1 receptor efficacy. Eur J Pharmacol 188: 335-347

Anderson JW (1954) The production of ultrasonic sounds by laboratory rats and other mammals. Science 119: 808-809

Aragona BJ, Cleaveland NA, Stuber GD, Day JJ, Carelli RM, Wightman RM (2008) Preferential enhancement of dopamine transmission within the nucleus accumbens shell by cocaine is attributable to a direct increase in phasic dopamine release events. J Neurosci 28: 8821-8831

Arnold JC, Dielenberg RA, McGregor IS (2010) Cannabinoids increase conditioned ultrasonic vocalisations and cat odour avoidance in rats: strain differences in drug-induced anxiety. Life Sci 87: 572-578

Badrinarayan A, Wescott SA, Vander Weele CM, Saunders BT, Couturier BE, Maren S, Aragona BJ (2012) Aversive Stimuli Differentially Modulate Real-Time Dopamine Transmission Dynamics within the Nucleus Accumbens Core and Shell. J Neurosci 32: 15779-15790

Bamann C, Kirsch T, Nagel G, Bamberg E (2008) Spectral characteristics of the photocycle of channelrhodopsin-2 and its implication for channel function. J Mol Biol 375: 686-694

Banasikowski TJ, Beninger RJ (2012) Reduced expression of haloperidol conditioned catalepsy in rats by the dopamine D3 receptor antagonists nafadotride and NGB 2904. Eur Neuropsychopharmacol 22: 761-768

Bardo MT, Neisewander JL, Kelly TH (2013) Individual differences and social influences on the neurobehavioral pharmacology of abused drugs. Pharmacol Rev 65: 255-290

Barfield RJ, Geyer LA (1972) Sexual behavior: ultrasonic postejaculatory song of the male rat. Science 176: 1349-1350

Barker DJ, Herrera C, West OW (2014a) Automated detection of 50-kHz ultrasonic vocalizations using template matching in XBAT. J Neurosci Methods 236: 68-75

Barker DJ, Root DH, Ma S, Jha S, Megehee L, Pawlak AP, West MO (2010) Dose-dependent differences in short ultrasonic vocalizations emitted by rats during cocaine self-administration. Psychopharmacology (Berl) 211: 435-442

Barker DJ, Simmons SJ, Servilio LC, Bercovicz D, Ma S, Root DH, Pawlak AP, West MO (2014b) Ultrasonic vocalizations: evidence for an affective opponent process during cocaine self-administration. Psychopharmacology (Berl) 231: 909-918

Bartoszyk GD (1998) Anxiolytic effects of dopamine receptor ligands: I. Involvement of dopamine autoreceptors. Life Sci 62: 649-663

Bass CE, Grinevich VP, Kulikova AD, Bonin KD, Budygin EA (2013) Terminal effects of optogenetic stimulation on dopamine dynamics in rat striatum. J Neurosci Methods 214: 149-155

Beaulieu JM, Gainetdinov RR (2011) The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev 63: 182-217

Berger AL, Williams AM, McGinnis MM, Walker BM (2013) Affective cue-induced escalation of alcohol self-administration and increased 22-kHz ultrasonic vocalizations during alcohol withdrawal: role of kappa-opioid receptors. Neuropsychopharmacology 38: 647-654

Berridge CW, Stalnaker TA (2002) Relationship between low-dose amphetamine-induced arousal and extracellular norepinephrine and dopamine levels within prefrontal cortex. Synapse 46: 140-149

Berridge KC, Kringelbach ML (2008) Affective neuroscience of pleasure: reward in humans and animals. Psychopharmacology (Berl) 199: 457-480

Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 28: 309-369

Berridge KC, Robinson TE, Aldridge JW (2009) Dissecting components of reward: 'liking', 'wanting', and learning. Curr Opin Pharmacol 9: 65-73

Bialy M, Kalata U, Nikolaev-Diak A, Nikolaev E (2010) D1 receptors involved in the acquisition of sexual experience in male rats. Behav Brain Res 206: 166-176

bi-Dargham A (2014) Schizophrenia: overview and dopamine dysfunction. J Clin Psychiatry 75: e31

Binkley KA, Webber ES, Powers DD, Cromwell HC (2014) Emotion and relative reward processing: An investigation on instrumental successive negative contrast and ultrasonic vocalizations in the rat. Behav Processes 107C: 167-174

Blanchard RJ, Agullana R, McGee L, Weiss S, Blanchard DC (1992) Sex differences in the incidence and sonographic characteristics of antipredator ultrasonic cries in the laboratory rat (Rattus norvegicus). J Comp Psychol 106: 270-277

Blanchard RJ, Blanchard DC, Agullana R, Weiss SM (1991) Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. Physiol Behav 50: 967-972

Blesa J, Przedborski S (2014) Parkinson's disease: animal models and dopaminergic cell vulnerability. Front Neuroanat 8: 155

Blumberg MS, Johnson ED, Middlemis-Brown JE (2005) Inhibition of ultrasonic vocalizations by beta-adrenoceptor agonists. Dev Psychobiol 47: 66-76

Bogdanova OV, Kanekar S, D'Anci KE, Renshaw PF (2013) Factors influencing behavior in the forced swim test. Physiol Behav 118: 227-239

Boulougouris V, Castane A, Robbins TW (2009) Dopamine D2/D3 receptor agonist quinpirole impairs spatial reversal learning in rats: investigation of D3 receptor involvement in persistent behavior. Psychopharmacology (Berl) 202: 611-620

Bowery BJ, Razzaque Z, Emms F, Patel S, Freedman S, Bristow L, Kulagowski J, Seabrook GR (1996) Antagonism of the effects of (+)-PD 128907 on midbrain dopamine neurones in rat brain slices by a selective D2 receptor antagonist L-741,626. Br J Pharmacol 119: 1491-1497

Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K (2005) Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci 8: 1263-1268

Brainard MS, Fitch WT (2014) Editorial overview: communication and language: animal communication and human language. Curr Opin Neurobiol 28: v-viii

Brenes JC, Schwarting RK (2014) Attribution and expression of incentive salience are differentially signaled by ultrasonic vocalizations in rats. PLoS One 9: e102414

Brenes JC, Schwarting RK (2015) Individual differences in anticipatory activity to food rewards predict cue-induced appetitive 50-kHz calls in rats. Physiol Behav 9: e102414

Breton YA, Marcus JC, Shizgal P (2009) Rattus Psychologicus: construction of preferences by self-stimulating rats. Behav Brain Res 202: 77-91

Britt JP, McDevitt RA, Bonci A (2012) Use of channelrhodopsin for activation of CNS neurons. Curr Protoc Neurosci Chapter 2: Unit2

Bromberg-Martin ES, Matsumoto M, Hikosaka O (2010) Dopamine in motivational control: rewarding, aversive, and alerting. Neuron 68: 815-834

Brown HD, McCutcheon JE, Cone JJ, Ragozzino ME, Roitman MF (2011) Primary food reward and reward-predictive stimuli evoke different patterns of phasic dopamine signaling throughout the striatum. Eur J Neurosci 34: 1997-2006

Browning JR, Browning DA, Maxwell AO, Dong Y, Jansen HT, Panksepp J, Sorg BA (2011) Positive affective vocalizations during cocaine and sucrose self-administration: a model for spontaneous drug desire in rats. Neuropharmacology 61: 268-275

Brudzynski SM (2005) Principles of rat communication: quantitative parameters of ultrasonic calls in rats. Behav Genet 35: 85-92

Brudzynski SM (2007) Ultrasonic calls of rats as indicator variables of negative or positive states: acetylcholine-dopamine interaction and acoustic coding. Behav Brain Res 182: 261-273

Brudzynski SM (2013) Ethotransmission: communication of emotional states through ultrasonic vocalization in rats. Curr Opin Neurobiol 23: 310-317

Brudzynski SM, Barnabi F (1996) Contribution of the ascending cholinergic pathways in the production of ultrasonic vocalization in the rat. Behav Brain Res 80: 145-152

Brudzynski SM, Bihari F (1990) Ultrasonic vocalization in rats produced by cholinergic stimulation of the brain. Neurosci Lett 109: 222-226

Brudzynski SM, Gibson B, Silkstone M, Burgdorf J, Kroes RA, Moskal JR, Panksepp J (2011) Motor and locomotor responses to systemic amphetamine in three lines of selectively bred Long-Evans rats. Pharmacol Biochem Behav 100: 119-124

Brudzynski SM, Komadoski M, St Pierre J (2012) Quinpirole-induced 50kHz ultrasonic vocalization in the rat: Role of D2 and D3 dopamine receptors. Behav Brain Res 511-518

Brudzynski SM, Pniak A (2002) Social contacts and production of 50-kHz short ultrasonic calls in adult rats. J Comp Psychol 116: 73-82

Buck CL, Malavar JC, George O, Koob GF, Vendruscolo LF (2014a) Anticipatory 50 kHz ultrasonic vocalizations are associated with escalated alcohol intake in dependent rats. Behav Brain Res 271: 171-176

Buck CL, Vendruscolo LF, Koob GF, George O (2014) Dopamine D1 and mu-opioid receptor antagonism blocks anticipatory 50 kHz ultrasonic vocalizations induced by palatable food cues in Wistar rats. Psychopharmacology (Berl) 231: 929-937

Burgdorf J, Knutson B, Panksepp J (2000) Anticipation of rewarding electrical brain stimulation evokes ultrasonic vocalization in rats. Behav Neurosci 114: 320-327

Burgdorf J, Knutson B, Panksepp J, Ikemoto S (2001) Nucleus accumbens amphetamine microinjections unconditionally elicit 50-kHz ultrasonic vocalizations in rats. Behav Neurosci 115: 940-944

Burgdorf J, Knutson B, Panksepp J, Ikemoto S (2001a) Nucleus accumbens amphetamine microinjections unconditionally elicit 50-kHz ultrasonic vocalizations in rats. Behav Neurosci 115: 940-944

Burgdorf J, Kroes RA, Moskal JR, Pfaus JG, Brudzynski SM, Panksepp J (2008) Ultrasonic vocalizations of rats (Rattus norvegicus) during mating, play, and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback. J Comp Psychol 122: 357-367

Burgdorf J, Panksepp J, Brudzynski SM, Beinfeld MC, Cromwell HC, Kroes RA, Moskal JR (2009) The effects of selective breeding for differential rates of 50-kHz ultrasonic vocalizations on emotional behavior in rats. Dev Psychobiol 51: 34-46

Burgdorf J, Panksepp J, Moskal JR (2011) Frequency-modulated 50 kHz ultrasonic vocalizations: a tool for uncovering the molecular substrates of positive affect. Neurosci Biobehav Rev 35: 1831-1836

Burgdorf J, Wood PL, Kroes RA, Moskal JR, Panksepp J (2007) Neurobiology of 50-kHz ultrasonic vocalizations in rats: electrode mapping, lesion, and pharmacology studies. Behav Brain Res 182: 274-283

Cagiano R, Barfield RJ, White NR, Pleim ET, Cuomo V (1989) Mediation of rat postejaculatory 22 kHz ultrasonic vocalization by dopamine D2 receptors. Pharmacol Biochem Behav 34: 53-58

Calvino B, Besson JM, Boehrer A, Depaulis A (1996) Ultrasonic vocalization (22-28 kHz) in a model of chronic pain, the arthritic rat: effects of analgesic drugs. Neuroreport 7: 581-584

Cameron CM, Wightman RM, Carelli RM (2014) Dynamics of rapid dopamine release in the nucleus accumbens during goal-directed behaviors for cocaine versus natural rewards. Neuropharmacology 86: 319-328

Capper-Loup C, Canales JJ, Kadaba N, Graybiel AM (2002) Concurrent activation of dopamine D1 and D2 receptors is required to evoke neural and behavioral phenotypes of cocaine sensitization. J Neurosci 22: 6218-6227

Carlsson A, Lindqvist M, Magnusson T (1957) 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature 180: 1200

Carra G, Bartoli F, Crocamo C, Brady KT, Clerici M (2014) Attempted suicide in people with co-occurring bipolar and substance use disorders: systematic review and meta-analysis. J Affect Disord 167: 125-135

Chang WL, Breier MR, Yang A, Swerdlow NR (2011) Disparate effects of pramipexole on locomotor activity and sensorimotor gating in Sprague-Dawley rats. Pharmacol Biochem Behav

99: 634-638

Cheer JF, Heien ML, Garris PA, Carelli RM, Wightman RM (2005) Simultaneous dopamine and single-unit recordings reveal accumbens GABAergic responses: implications for intracranial self-stimulation. Proc Natl Acad Sci U S A 102: 19150-19155

Cheer JF, Wassum KM, Heien ML, Phillips PE, Wightman RM (2004) Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. J Neurosci 24: 4393-4400

Cheer JF, Wassum KM, Sombers LA, Heien ML, Ariansen JL, Aragona BJ, Phillips PE, Wightman RM (2007) Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. J Neurosci 27: 791-795

Chehayeb, D. (2007) Factors affecting amphetamine-induced 50 kHz ultrasonic vocalizations in adult rats. Master thesis, McGill University

Chenu F, Shim S, El MM, Blier P (2014) Role of melatonin, serotonin 2B, and serotonin 2C receptors in modulating the firing activity of rat dopamine neurons. J Psychopharmacol 28: 162-167

Chergui K, Suaud-Chagny MF, Gonon F (1994) Nonlinear relationship between impulse flow, dopamine release and dopamine elimination in the rat brain in vivo. Neuroscience 62: 641-645

Chiu WT, Lin CM, Tsai TC, Wu CW, Tsai CL, Lin SH, Chen JJ (2014) Real-time electrochemical recording of dopamine release under optogenetic stimulation PLoS One 9: e89293

Chow BY, Han X, Dobry AS, Qian X, Chuong AS, Li M, Henninger MA, Belfort GM, Lin Y, Monahan PE, Boyden ES (2010) High-performance genetically targetable optical neural silencing by light-driven proton pumps. Nature 463: 98-102

Ciucci MR, Ahrens AM, Ma ST, Kane JR, Windham EB, Woodlee MT, Schallert T (2009) Reduction of dopamine synaptic activity: degradation of 50-kHz ultrasonic vocalization in rats. Behav Neurosci 123: 328-336

Clark D, White FJ (1987) D1 dopamine receptor--the search for a function: a critical evaluation of the D1/D2 dopamine receptor classification and its functional implications. Synapse 1: 347-388

Clark JJ, Sandberg SG, Wanat MJ, Gan JO, Horne EA, Hart AS, Akers CA, Parker JG, Willuhn I, Martinez V, Evans SB, Stella N, Phillips PE (2010) Chronic microsensors for longitudinal, subsecond dopamine detection in behaving animals. Nat Methods 7: 126-129

Clarke PB, Wright JM (2015) Ultrasonic Vocalizations. In: Stolerman I, Price LH (eds) Encyclopedia of Psychopharmacology. Springer Berlin, Heidelberg, pp 1765-1771

Clifford JJ, Waddington JL (2000) Topographically based search for an "Ethogram" among a series of novel D(4) dopamine receptor agonists and antagonists. Neuropsychopharmacology 22: 538-544

Coffey KR, Barker DJ, Ma S, Root DH, Martinez L, Horvitz JC, West MO (2012) Effects of Varying Reinforcement Probability on Pavlovian Approach Behavior and Ultrasonic Vocalizationsin Rats. Behav Brain Res 237: 256-262

Collins GT, Newman AH, Grundt P, Rice KC, Husbands SM, Chauvignac C, Chen J, Wang S, Woods JH (2007) Yawning and hypothermia in rats: effects of dopamine D3 and D2 agonists and antagonists. Psychopharmacology (Berl) 193: 159-170

Corrigan JG, Flannelly KJ (1979) Ultrasonic vocalizations of defeated male rats. J Comp Phys Psych 93: 105-115

Cory-Slechta DA, Zuch CL, Fox RA (1996) Comparison of the stimulus properties of a pre- vs. a putative postsynaptic dose of quinpirole. Pharmacol Biochem Behav 55: 423-432

Costa G, Morelli M, Simola N (2015) Involvement of Glutamate NMDA Receptors in the Acute, Long-Term, and Conditioned Effects of Amphetamine on Rat 50kHz Ultrasonic Vocalizations. Int J Neuropsychopharmacol doi: 10.1093/ijnp/pyv057

Covey DP, Juliano SA, Garris PA (2013) Amphetamine elicits opposing actions on readily releasable and reserve pools for dopamine. PLoS One 8: e60763

Covey DP, Roitman MF, Garris PA (2014) Illicit dopamine transients: reconciling actions of abused drugs. Trends Neurosci 37: 200-210

Covington HE, III, Miczek KA (2003) Vocalizations during withdrawal from opiates and cocaine: possible expressions of affective distress. Eur J Pharmacol 467: 1-13

Cuomo V, Cagiano R (1987) Ultrasonic vocalization in rodents: a new potential tool for detecting emotional and motivational changes produced by adverse treatments. Zentralbl Bakteriol Mikrobiol Hyg B 185: 55-60

Cuomo V, Cagiano R, De Salvia MA, Maselli MA, Renna G, Racagni G (1988) Ultrasonic vocalization in response to unavoidable aversive stimuli in rats: effects of benzodiazepines. Life Sci 43: 485-491

Daberkow DP, Brown HD, Bunner KD, Kraniotis SA, Doellman MA, Ragozzino ME, Garris PA, Roitman MF (2013) Amphetamine paradoxically augments exocytotic dopamine release and phasic dopamine signals. J Neurosci 33: 452-463

Daglish MR, Nutt DJ (2003) Brain imaging studies in human addicts. Eur Neuropsychopharmacol 13: 453-458

Dall'olio R, Gandolfi O, Vaccheri A, Roncada P, Montanaro N (1988) Changes in behavioural responses to the combined administration of D1 and D2 dopamine agonists in normosensitive

and D1 supersensitive rats. Psychopharmacology (Berl) 95: 381-385

D'Aquila PS, Collu M, Pani L, Gessa GL, Serra G (1994) Antidepressant-like effect of selective dopamine D1 receptor agonists in the behavioural despair animal model of depression. Eur J Pharmacol 262: 107-111

Day JJ, Jones JL, Wightman RM, Carelli RM (2010) Phasic nucleus accumbens dopamine release encodes effort- and delay-related costs. Biol Psychiatry 68: 306-309

Day JJ, Roitman MF, Wightman RM, Carelli RM (2007) Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nat Neurosci 10: 1020-1028

De BM, Dani JA (2011) Reward, addiction, withdrawal to nicotine. Annu Rev Neurosci 34: 105-130

De VJ, Benz U, Schreiber R, Traber J (1993) Shock-induced ultrasonic vocalization in young adult rats: a model for testing putative anti-anxiety drugs. Eur J Pharmacol 249: 331-339

DeNinno MP, Schoenleber R, MacKenzie R, Britton DR, Asin KE, Briggs C, Trugman JM, Ackerman M, Artman L, Bednarz L (1991) A68930: a potent agonist selective for the dopamine D1 receptor. Eur J Pharmacol 199: 209-219

Deutch AY, Cameron DS (1992) Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. Neuroscience 46: 49-56

Deveney AM, Waddington JL (1997) Psychopharmacological distinction between novel full-efficacy "D1-like" dopamine receptor agonists. Pharmacol Biochem Behav 58: 551-558

Di Chiara G, Bassareo V (2007) Reward system and addiction: what dopamine does and doesn't do. Curr Opin Pharmacol 7: 69-76

Dinh HK, Larkin A, Gatlin L, Piepmeier E Jr (1999) Rat ultrasound model for measuring pain resulting from intramuscularly injected antimicrobials. PDA J Pharm Sci Technol 53: 40-43

Eddine R, Valverde S, Tolu S, Dautan D, Hay A, Morel C, Cui Y, Lambolez B, Venance L, Marti F, Faure P (2015) A concurrent excitation and inhibition of dopaminergic subpopulations in response to nicotine. Sci Rep 5: 8184

Einax JW (2006) Practical Guide to Chemometrics, 2 edn. Taylor and Francis Ltd.

Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8: 1481-1489

Fabbricatore AT, Ghitza UE, Prokopenko VF, West MO (2009) Electrophysiological evidence of mediolateral functional dichotomy in the rat accumbens during cocaine self-administration: tonic firing patterns. Eur J Neurosci 30: 2387-2400

Fabbricatore AT, Ghitza UE, Prokopenko VF, West MO (2010) Electrophysiological evidence of mediolateral functional dichotomy in the rat nucleus accumbens during cocaine self-administration II: phasic firing patterns. Eur J Neurosci 31: 1671-1682

Faure A, Richard JM, Berridge KC (2010) Desire and dread from the nucleus accumbens: cortical glutamate and subcortical GABA differentially generate motivation and hedonic impact in the rat. PLoS One 5: e11223

Fendt M, Schwienbacher I, Schnitzler HU (2006) Carbachol injections into the nucleus accumbens induce 50 kHz calls in rats. Neurosci Lett 401: 10-15

Fenu S, Rivas E, Di Chiara G (2005) Differential role of dopamine in drug- and lithium-conditioned saccharin avoidance. Physiol Behav 85: 37-43

Finn IB, Iuvone PM, Holtzman SG (1990) Depletion of catecholamines in the brain of rats differentially affects stimulation of locomotor activity by caffeine, D-amphetamine, and methylphenidate. Neuropharmacology 29: 625-631

Fiorillo CD, Tobler PN, Schultz W (2003) Discrete coding of reward probability and uncertainty by dopamine neurons. Science 299: 1898-1902

Flagel SB, Clark JJ, Robinson TE, Mayo L, Czuj A, Willuhn I, Akers CA, Clinton SM, Phillips PE, Akil H (2011) A selective role for dopamine in stimulus-reward learning. Nature 469: 53-57

Floresco SB, West AR, Ash B, Moore H, Grace AA (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. Nat Neurosci 6: 968-973

Fortin SM, Cone JJ, Ng-Evans S, McCutcheon JE, Roitman MF (2015) Sampling phasic dopamine signaling with fast-scan cyclic voltammetry in awake, behaving rats. Curr Protoc Neurosci 70: 7.25.1-7.25.20

Freeman J, Maxwell JC, Davey J (2011) Unraveling the complexity of driving while intoxicated: a study into the prevalence of psychiatric and substance abuse comorbidity. Accid Anal Prev 43: 34-39

Fu XW, Brudzynski SM (1994) High-frequency ultrasonic vocalization induced by intracerebral glutamate in rats. Pharmacol Biochem Behav 49: 835-841

Gan JO, Walton ME, Phillips PE (2010) Dissociable cost and benefit encoding of future rewards by mesolimbic dopamine. Nat Neurosci 13: 25-27

Garcia EJ, McCowan TJ, Cain ME (2015) Harmonic and frequency modulated ultrasonic vocalizations reveal differences in conditioned and unconditioned reward processing. Behav Brain Res 287: 207-214

Garrett BE, Holtzman SG (1994) Caffeine cross-tolerance to selective dopamine D1 and D2 receptor agonists but not to their synergistic interaction. Eur J Pharmacol 262: 65-75

Garris PA, Ciolkowski EL, Pastore P, Wightman RM (1994) Efflux of dopamine from the synaptic cleft in the nucleus accumbens of the rat brain. J Neurosci 14: 6084-6093

Garris PA, Kilpatrick M, Bunin MA, Michael D, Walker QD, Wightman RM (1999) Dissociation of dopamine release in the nucleus accumbens from intracranial self-stimulation. Nature 398: 67-69

Gehlert DR, Gackenheimer SL, Seeman P, Schaus J (1992) Autoradiographic localization of [3H]quinpirole binding to dopamine D2 and D3 receptors in rat brain. Eur J Pharmacol 211: 189-194

Glase SA, Akunne HC, Georgic LM, Heffner TG, MacKenzie RG, Manley PJ, Pugsley TA, Wise LD (1997) Substituted [(4-phenylpiperazinyl)-methyl]benzamides: selective dopamine D4 agonists. J Med Chem 40: 1771-1772

Glimcher PW (2011) Understanding dopamine and reinforcement learning: the dopamine reward prediction error hypothesis. Proc Natl Acad Sci USA 108 Suppl 3: 15647-15654

Goldman-Rakic PS (1995) Cellular basis of working memory. Neuron 14: 477-485

Gonon FG (1988) Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. Neuroscience 24: 19-28

Goto Y, Grace AA (2005) Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. Nat Neurosci 8: 805-812

Gould J, Morgan C (1941) Hearing in the rat at high frequencies. Science 94: 168

Grace AA, Bunney BS (1984a) The control of firing pattern in nigral dopamine neurons: single spike firing. J Neurosci 4: 2866-2876

Grace AA, Bunney BS (1984b) The control of firing pattern in nigral dopamine neurons: burst firing. J Neurosci 4: 2877-2890

Graham DL, Hoppenot R, Hendryx A, Self DW (2007) Differential ability of D1 and D2 dopamine receptor agonists to induce and modulate expression and reinstatement of cocaine place preference in rats. Psychopharmacology (Berl) 191: 719-730

Grant LM, Barnett DG, Doll EJ, Leverson G, Ciucci M (2015) Relationships among rat ultrasonic vocalizations, behavioral measures of striatal dopamine loss, and striatal tyrosine hydroxylase immunoreactivity at acute and chronic time points following unilateral 6-hydroxydopamine-induced dopamine depletion. Behav Brain Res 291: 361-371

Grecksch G, Matties H (1981) The role of dopaminergic mechanisms in the rat hippocampus for the consolidation in a brightness discrimination. Psychopharmacology (Berl) 75: 165-168

Green AS, Grahame NJ (2008) Ethanol drinking in rodents: is free-choice drinking related to the reinforcing effects of ethanol? Alcohol 42: 1-11

Gyertyan I, Gal K (2003) Dopamine D3 receptor ligands show place conditioning effect but do not influence cocaine-induced place preference. Neuroreport 14: 93-98

Gyertyan I, Saghy K (2004) Effects of dopamine D3 receptor antagonists on spontaneous and agonist-reduced motor activity in NMRI mice and Wistar rats: comparative study with nafadotride, U 99194A and SB 277011. Behav Pharmacol 15: 253-262

Hamdani S, White NM (2011) Ultrasonic vocalization ratios reflect the influence of motivational state and amygdala lesions on different types of taste avoidance learning. Behav Brain Res 217: 88-98

Hamed A, Szyndler J, Taracha E, Turzynska D, Sobolewska A, Lehner M, Krzascik P, Daszczuk P (2015) kappa-opioid receptor as a key mediator in the regulation of appetitive 50-kHz ultrasonic vocalizations. Psychopharmacology (Berl) 232: 1941-1955

Hammen C (2005) Stress and depression. Annu Rev Clin Psychol 1: 293-319

Han X, Boyden ES (2007) Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution. PLoS One 2: e299

Haney M, Miczek KA (1993) Ultrasounds during agonistic interactions between female rats (Rattus norvegicus). J Comp Psychol 107: 373-379

Hart AS, Rutledge RB, Glimcher PW, Phillips PE (2014) Phasic dopamine release in the rat nucleus accumbens symmetrically encodes a reward prediction error term. J Neurosci 34: 698-704

Hasbi A, O'Dowd BF, George SR (2011) Dopamine D1-D2 receptor heteromer signaling pathway in the brain: emerging physiological relevance. Mol Brain 4: 26

Heidbreder C, Feldon J (1998) Amphetamine-induced neurochemical and locomotor responses are expressed differentially across the anteroposterior axis of the core and shell subterritories of the nucleus accumbens. Synapse 29: 310-322

Heien ML, Johnson MA, Wightman RM (2004) Resolving neurotransmitters detected by fast-scan cyclic voltammetry. Anal Chem 76: 5697-5704

Heien ML, Khan AS, Ariansen JL, Cheer JF, Phillips PE, Wassum KM, Wightman RM (2005) Real-time measurement of dopamine fluctuations after cocaine in the brain of behaving rats. Proc Natl Acad Sci U S A 102: 10023-10028

Heien ML, Phillips PE, Stuber GD, Seipel AT, Wightman RM (2003) Overoxidation of carbon-fiber microelectrodes enhances dopamine adsorption and increases sensitivity. Analyst 128: 1413-1419

Heimer L, Alheid GF, De Olmos JS, Groenewegen HJ, Haber SN, Harlan RE, Zahm DS (1997) The accumbens: beyond the core-shell dichotomy. J Neuropsychiatry Clin Neurosci 9: 354-381

Heyse NC, Brenes JC, Schwarting RK (2015) Exercise reward induces appetitive 50-kHz calls in rats. Physiol Behav 147: 131-140

Himmler BT, Kisko TM, Euston DR, Kolb B, Pellis SM (2014a) Are 50-kHz calls used as play signals in the playful interactions of rats? I. Evidence from the timing and context of their use. Behav Processes 106: 60-66

Hoffman DC, Beninger RJ (1988) Selective D1 and D2 dopamine agonists produce opposing effects in place conditioning but not in conditioned taste aversion learning. Pharmacol Biochem Behav 31: 1-8

Hollis F, Kabbaj M (2014) Social defeat as an animal model for depression. ILAR J 55: 221-232

Hollon NG, Arnold MM, Gan JO, Walton ME, Phillips PE (2014) Dopamine-associated cached values are not sufficient as the basis for action selection. Proc Natl Acad Sci U S A 111: 18357-18362

Hori M, Shimoju R, Tokunaga R, Ohkubo M, Miyabe S, Ohnishi J, Murakami K, Kurosawa M (2013) Tickling increases dopamine release in the nucleus accumbens and 50 kHz ultrasonic vocalizations in adolescent rats. Neuroreport 24: 241-245

Horvitz JC, Williams G, Joy R (2001) Time-dependent actions of D2 family agonist quinpirole on spontaneous behavior in the rat: dissociation between sniffing and locomotion. Psychopharmacology (Berl) 154: 350-355

Howe MW, Tierney PL, Sandberg SG, Phillips PE, Graybiel AM (2013) Prolonged dopamine signalling in striatum signals proximity and value of distant rewards. Nature 500: 575-579

Hsieh GC, Hollingsworth PR, Martino B, Chang R, Terranova MA, O'Neill AB, Lynch JJ, Moreland RB, Donnelly-Roberts DL, Kolasa T, Mikusa JP, McVey JM, Marsh KC, Sullivan JP, Brioni JD (2004) Central mechanisms regulating penile erection in conscious rats: the dopaminergic systems related to the proerectile effect of apomorphine. J Pharmacol Exp Ther 308: 330-338

Ikemoto S (2002) Ventral striatal anatomy of locomotor activity induced by cocaine, D-amphetamine, dopamine and D1/D2 agonists. Neuroscience 113: 939-955

Ikemoto S (2007) Dopamine reward circuitry: two projections systems from ventral midbrain to the nucleus accumbens-olfactory tubercle complex. Brain Res Rev 56: 27-78

Ikemoto S (2010) Brain reward circuitry beyond the mesolimbic dopamine system: a neurobiological theory. Neurosci Biobehav Rev 35: 129-150

Ikemoto S, Glazier BS, Murphy JM, McBride WJ (1997) Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. J Neurosci 17: 8580-8587

Ilango A, Kesner AJ, Broker CJ, Wang DV, Ikemoto S (2014a) Phasic excitation of ventral tegmental dopamine neurons potentiates the initiation of conditioned approach behavior: parametric and reinforcement-schedule analyses. Front Behav Neurosci 8: 155

Ilango A, Kesner AJ, Keller KL, Stuber GD, Bonci A, Ikemoto S (2014b) Similar roles of substantia nigra and ventral tegmental dopamine neurons in reward and aversion. J Neurosci 34: 817-822

Inagaki H, Kuwahara M, Kikusui T, Tsubone H (2005) The influence of social environmental condition on the production of stress-induced 22 kHz calls in adult male Wistar rats. Physiol Behav 84: 17-22

Isacson R, Kull B, Wahlestedt C, Salmi P (2004) A 68930 and dihydrexidine inhibit locomotor activity and d-amphetamine-induced hyperactivity in rats: a role of inhibitory dopamine D(1/5) receptors in the prefrontal cortex? Neuroscience 124: 33-42

Jackson DM, Westlind-Danielsson A (1994) Dopamine receptors: molecular biology, biochemistry and behavioural aspects. Pharmacol Ther 64: 291-370

Johnson AM, Ciucci MR, Russell JA, Hammer MJ, Connor NP (2010) Ultrasonic output from the excised rat larynx. J Acoust Soc Am 128: EL75-EL79

Jones CA, Watson DJ, Fone KC (2011) Animal models of schizophrenia. Br J Pharmacol 164: 1162-1194

Joyce EM, Iversen SD (1984) Dissociable effects of 6-OHDA-induced lesions of neostriatum on anorexia, locomotor activity and stereotypy: the role of behavioural competition. Psychopharmacology (Berl) 83: 363-366

Kalivas PW, Bourdelais A, Abhold R, Abbott L (1989) Somatodendritic release of endogenous dopamine: in vivo dialysis in the A10 dopmaine region. Neurosci Lett 100: 215-20

Kaltwasser MT (1990) Acoustic signaling in the black rat (Rattus rattus). J Comp Psychol 104: 227-232

Kashtelyan V, Lichtenberg NT, Chen ML, Cheer JF, Roesch MR (2014) Observation of reward delivery to a conspecific modulates dopamine release in ventral striatum. Curr Biol 24: 2564-2568

Kebabian JW, DeNinno MP, Schoenleber R, MacKenzie R, Britton DR, Asin KE (1992) A68930: a potent agonist specific for the dopamine D1 receptor. Neurochem Int 20 Suppl: 157S-160S

Keefe KA, Zigmond MJ, Abercrombie ED (1993) In vivo regulation of extracellular dopamine in the neostriatum: influence of impulse activity and local excitatory amino acids. J Neural Transm Gen Sect 91: 223-240

Keithley RB, Heien ML, Wightman RM (2009) Multivariate concentration determination using principal component regression with residual analysis. Trends Analyt Chem 28: 1127-1136

Kershenbaum A, Bowles AE, Freeberg TM, Jin DZ, Lameira AR, Bohn K (2014) Animal vocal sequences: not the Markov chains we thought they were. Proc Biol Sci 281: 1-9

Khroyan TV, Fuchs RA, Baker DA, Neisewander JL (1997) Effects of D3-preferring agonists 7-OH-PIPAT and PD-128,907 on motor behaviors and place conditioning. Behav Pharmacol 8: 65-74

Kilpatrick MR, Rooney MB, Michael DJ, Wightman RM (2000) Extracellular dopamine dynamics in rat caudate-putamen during experimenter-delivered and intracranial self-stimulation. Neuroscience 96: 697-706

Kim EJ, Horovitz O, Pellman BA, Tan LM, Li Q, Richter-Levin G, Kim JJ (2013) Dorsal periaqueductal gray-amygdala pathway conveys both innate and learned fear responses in rats. Proc Natl Acad Sci U S A 110: 14795-14800

Kirby, M. and Keon, W. Mental health, mental illness and addiction: Overview of policies and programs in Canada. 1. 2004. Interim report of the Standing Senate Committee on Social Affairs, Science and Technology.

Kisko TM, Himmler BT, Himmler SM, Euston DR, Pellis SM (2015) Are 50-kHz calls used as play signals in the playful interactions of rats? II. Evidence from the effects of devocalization. Behav Processes 111: 25-33

Kleiman GH, Barnea A, Gozes I (2015) ADNP: A major autism mutated gene is differentially distributed (age and gender) in the songbird brain. Peptides doi: 10.1016/j.peptides.2015.04.008

Knapp DJ, Pohorecky LA (1995) An air-puff stimulus method for elicitation of ultrasonic vocalizations in rats. J Neurosci Methods 62: 1-5

Knutson B, Burgdorf J, Panksepp J (1998) Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats. J Comp Psychol 112: 65-73

Knutson B, Burgdorf J, Panksepp J (2002) Ultrasonic vocalizations as indices of affective states in rats. Phsychol Bull 128: 961-977

Koffarnus MN, Newman AH, Grundt P, Rice KC, Woods JH (2011) Effects of selective dopaminergic compounds on a delay-discounting task. Behav Pharmacol 22: 300-311

Kozai TD, Li X, Bodily LM, Caparosa EM, Zenonos GA, Carlisle DL, Friedlander RM, Cui XT (2014) Effects of caspase-1 knockout on chronic neural recording quality and longevity: insight into cellular and molecular mechanisms of the reactive tissue response. Biomaterials 35: 9620-9634

Kriengwatana B, Escudero P, Ten CC (2014) Revisiting vocal perception in non-human animals: a review of vowel discrimination, speaker voice recognition, and speaker normalization. Front Psychol 5: 1543

Kuczenski R, Melega WP, Cho AK, Segal DS (1997) Extracellular dopamine and amphetamine after systemic amphetamine administration: comparison to the behavioral response. J Pharmacol Exp Ther 282: 591-596

Kuczenski R, Segal DS, Cho AK, Melega W (1995) Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. J Neurosci 15: 1308-1317

Kuhn KU, Meyer K, Maier W (2000) Flupenthixol- a partial atypical neuroleptic? Fortschr Neurol Psychiatr 68: S38-41

Kurniawan IT, Guitart-Masip M, Dolan RJ (2011) Dopamine and effort-based decision making. Front Neurosci 5: 81

LaHoste GJ, Henry BL, Marshall JF (2000) Dopamine D1 receptors synergize with D2, but not D3 or D4, receptors in the striatum without the involvement of action potentials. J Neurosci 20: 6666-6671

Lammel S, Lim BK, Ran C, Huang KW, Betley MJ, Tye KM, Deisseroth K, Malenka RC (2012) Input-specific control of reward and aversion in the ventral tegmental area. Nature 7423: 212-217

Lammel S, Tye KM, Warden MR (2014) Progress in understanding mood disorders: optogenetic dissection of neural circuits. Genes Brain Behav 13: 38-51

Latagliata EC, Valzania A, Pascucci T, Campus P, Cabib S, Puglisi-Allegra S (2014) Stress-induced activation of ventral tegmental mu-opioid receptors reduces accumbens dopamine tone by enhancing dopamine transmission in the medial pre-frontal cortex. Psychopharmacology (Berl) 231: 4099-4108

Levant B, Grigoriadis DE, DeSouza EB (1993) [3H]quinpirole binding to putative D2 and D3 dopamine receptors in rat brain and pituitary gland: a quantitative autoradiographic study. J Pharmacol Exp Ther 264: 991-1001

Leyton M, Vezina P (2014) Dopamine ups and downs in vulnerability to addictions: a neurodevelopmental model. Trends Pharmacol Sci 35: 268-276

Lin RE, Ambler L, Billingslea EN, Suh J, Batheja S, Tatard-Leitman V, Featherstone RE, Siegel SJ (2013) Electroencephalographic and early communicative abnormalities in Brattleboro rats. Physiol Rep 1: e00100

Litvin Y, Blanchard DC, Blanchard RJ (2007) Rat 22 kHz ultrasonic vocalizations as alarm cries. Behav Brain Res 182: 166-172

Lopuch S, Popik P (2011) Cooperative behavior of laboratory rats (Rattus norvegicus) in an instrumental task. J Comp Psychol 125: 250-253

Lorenz-Fonfria VA, Heberle J (2014) Channelrhodopsin unchained: structure and mechanism of a light-gated cation channel. Biochim Biophys Acta 1837: 626-642

Lovell PV, Olson CR, Mello CV (2011) Singing under the influence: examining the effects of nutrition and addiction on a learned vocal behavior. Mol Neurobiol 44: 175-184

Ludvig EA, Conover K, Shizgal P (2007) The effects of reinforcer magnitude on timing in rats. J Exp Anal Behav 87: 201-218

Lynch WJ, Nicholson KL, Dance ME, Morgan RW, Foley PL (2010) Animal models of substance abuse and addiction: implications for science, animal welfare, and society. Comp Med 60: 177-188

Ma ST, Maier EY, Ahrens AM, Schallert T, Duvauchelle CL (2010) Repeated intravenous cocaine experience: development and escalation of pre-drug anticipatory 50-kHz ultrasonic vocalizations in rats. Behav Brain Res 212: 109-114

Mahler SV, Moorman DE, Feltenstein MW, Cox BM, Ogburn KB, Bachar M, McGonigal JT, Ghee SM, See RE (2013) A rodent "self-report" measure of methamphetamine craving? Rat ultrasonic vocalizations during methamphetamine self-administration, extinction, and reinstatement. Behav Brain Res 236: 78-89

Mallo T, Matrov D, Koiv K, Harro J (2009) Effect of chronic stress on behavior and cerebral oxidative metabolism in rats with high or low positive affect. Neuroscience 164: 963-974

Manduca A, Servadio M, Campolongo P, Palmery M, Trabace L, Vanderschuren LJ, Cuomo V, Trezza V (2014) Strain- and context-dependent effects of the anandamide hydrolysis inhibitor URB597 on social behavior in rats. Eur Neuropsychopharmacol 24: 1337-1348

Margolis EB, Hjelmstad GO, Fujita W, Fields HL (2014) Direct bidirectional mu-opioid control of midbrain dopamine neurons. J Neurosci 34: 14707-14716

Marinelli M, McCutcheon JE (2014) Heterogeneity of dopamine neuron activity across traits and states. Neuroscience 282C: 176-197

Mark GP, Shabani S, Dobbs LK, Hansen ST (2011) Cholinergic modulation of mesolimbic dopamine function and reward. Physiol Behav 104: 76-81

Masamizu Y, Okada T, Kawasaki K, Ishibashi H, Yuasa S, Takeda S, Hasegawa I, Nakahara K (2011) Local and retrograde gene transfer into primate neuronal pathways via adeno-associated virus serotype 8 and 9. Neuroscience 193: 249-258

Matheson LE, Sun H, Sakata JT (2015) Forebrain circuits underlying the social modulation of vocal communication signals. Dev Neurobiol doi: 10.1002/dneu.22298

McCue MG, LeDoux JE, Cain CK (2014) Medial amygdala lesions selectively block aversive pavlovian-instrumental transfer in rats. Front Behav Neurosci 8: 329

McCutcheon JE, Cone JJ, Sinon CG, Fortin SM, Kantak PA, Witten IB, Deisseroth K, Stuber GD, Roitman MF (2014) Optical suppression of drug-evoked phasic dopamine release. Front Neural Circuits 8: 114

McCutcheon JE, Ebner SR, Loriaux AL, Roitman MF (2012) Encoding of aversion by dopamine and the nucleus accumbens. Front Neurosci 6: 137

McLellan AT, Lewis DC, O'Brien CP, Kleber HD (2000) Drug dependence, a chronic medical illness: implications for treatment, insurance, and outcomes evaluation. JAMA 284: 1689-1695

McQuade RD, Duffy RA, Anderson CC, Crosby G, Coffin VL, Chipkin RE, Barnett A (1991) [3H]SCH 39166, a new D1-selective radioligand: in vitro and in vivo binding analyses. J Neurochem 57: 2001-2010

Melchior JR, Ferris MJ, Stuber GD, Riddle DR, Jones SR (2015) Optogenetic versus electrical stimulation of dopamine terminals in the nucleus accumbens reveals local modulation of presynaptic release. J Neurochem doi: 10.1111/jnc.13177

Melis MR, Succu S, Sanna F, Melis T, Mascia MS, Enguehard-Gueiffier C, Hubner H, Gmeiner P, Gueiffier A, Argiolas A (2006) PIP3EA and PD-168077, two selective dopamine D4 receptor agonists, induce penile erection in male rats: site and mechanism of action in the brain. Eur J Neurosci 24: 2021-2030

Mental Health Commission of Canada. Why investing in mental health will contribute to Canada's economic prosperity and to the sustainability of our health care system. 1-4. 2011.

Meredith GE, Agolia R, Arts MP, Groenewegen HJ, Zahm DS (1992) Morphological differences between projection neurons of the core and shell in the nucleus accumbens of the rat. Neuroscience 50: 149-162

Meredith GE, Baldo BA, Andrezjewski ME, Kelley AE (2008) The structural basis for mapping behavior onto the ventral striatum and its subdivisions. Brain Struct Funct 213: 17-27

Meyer PJ, Ma ST, Robinson TE (2011) A cocaine cue is more preferred and evokes more frequency-modulated 50-kHz ultrasonic vocalizations in rats prone to attribute incentive salience to a food cue. Psychopharmacology (Berl) 4: 999-1009

Miczek KA, Weerts EM, Vivian JA, Barros HM (1995) Aggression, anxiety and vocalizations in animals: GABAA and 5-HT anxiolytics. Psychopharmacology (Berl) 121: 38-56

Millan MJ, Brocco M, Papp M, Serres F, La Rochelle CD, Sharp T, Peglion JL, Dekeyne A (2004a) S32504, a novel naphtoxazine agonist at dopamine D3/D2 receptors: III. Actions in models of potential antidepressive and anxiolytic activity in comparison with ropinirole. J Pharmacol Exp Ther 309: 936-950

Millan MJ, Dekeyne A, Rivet JM, Dubuffet T, Lavielle G, Brocco M (2000) S33084, a novel, potent, selective, and competitive antagonist at dopamine D(3)-receptors: II. Functional and behavioral profile compared with GR218,231 and L741,626. J Pharmacol Exp Ther 293: 1063-1073

Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. J Pharmacol Exp Ther 303: 791-804

Millan MJ, Newman-Tancredi A, Brocco M, Gobert A, Lejeune F, Audinot V, Rivet JM, Schreiber R, Dekeyne A, Spedding M, Nicolas JP, Peglion JL (1998) S 18126 ([2-[4-(2,3-dihydrobenzo[1,4]dioxin-6-yl)piperazin-1-yl methyl]indan-2-yl]), a potent, selective and competitive antagonist at dopamine D4 receptors: an in vitro and in vivo comparison with L 745,870 (3-(4-[4-chlorophenyl]piperazin-1-yl)methyl-1H-pyrrolo[2, 3b]pyridine) and raclopride. J Pharmacol Exp Ther 287: 167-186

Millan MJ, Seguin L, Gobert A, Cussac D, Brocco M (2004b) The role of dopamine D3 compared with D2 receptors in the control of locomotor activity: a combined behavioural and neurochemical analysis with novel, selective antagonists in rats. Psychopharmacology (Berl) 174: 341-357

Miller S, Dell'Osso B, Ketter TA (2014) The prevalence and burden of bipolar depression. J Affect Disord 169 Suppl 1: S3-11

Mitrano DA, Schroeder JP, Smith Y, Cortright JJ, Bubula N, Vezina P, Weinshenker D (2012) Alpha-1 Adrenergic Receptors are Localized on Presynaptic Elements in the Nucleus Accumbens and Regulate Mesolimbic Dopamine Transmission. Neuropsychopharmacology 37: 2161-72

Moquin KF, Michael AC (2009) Tonic autoinhibition contributes to the heterogeneity of evoked dopamine release in the rat striatum. J Neurochem 110: 1491-1501

Moussy F, Harrison DJ (1994) Prevention of the rapid degradation of subcutaneously implanted Ag/AgCl reference electrodes using polymer coatings. Anal Chem 66: 674-679

Mu P, Fuchs T, Saal DB, Sorg BA, Dong Y, Panksepp J (2009) Repeated cocaine exposure induces sensitization of ultrasonic vocalization in rats. Neurosci Lett 453: 31-35

Natusch C, Schwarting RK (2010) Using bedding in a test environment critically affects 50-kHz ultrasonic vocalizations in laboratory rats. Pharmacol Biochem Behav 96: 251-259

Nergardh R, Oerther S, Fredholm BB (2005) Differences between A 68930 and SKF 82958 could suggest synergistic roles of D1 and D5 receptors. Pharmacol Biochem Behav 82: 495-505

Newby JM, McKinnon A, Kuyken W, Gilbody S, Dalgleish T (2015) Systematic review and meta-analysis of transdiagnostic psychological treatments for anxiety and depressive disorders in adulthood. Clin Psychol Rev 40: 91-110

Newman-Tancredi A, Cussac D, Quentric Y, Touzard M, Verriele L, Carpentier N, Millan MJ (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. III. Agonist and antagonist properties at serotonin, 5-HT(1) and 5-HT(2), receptor subtypes. J Pharmacol Exp Ther 303: 815-822

Niel L, Weary DN (2006) Behavioural responses of rats to gradual-fill carbon dioxide euthanasia and reduced oxygen concentrations. Appl Ani Beh Sci 100: 295-308

Niv Y (2007) Cost, benefit, tonic, phasic: what do response rates tell us about dopamine and motivation? Ann N Y Acad Sci 1104: 357-376

Oldenziel WH, Westerink BH (2005) Improving glutamate microsensors by optimizing the composition of the redox hydrogel. Anal Chem 77: 5520-5528

Opiol H, Pavlovski I, Michalik M, Mistlberger RE (2015) Ultrasonic vocalizations in rats anticipating circadian feeding schedules. Behav Brain Res 284: 42-50

Owesson-White CA, Ariansen J, Stuber GD, Cleaveland NA, Cheer JF, Wightman RM, Carelli RM (2009) Neural encoding of cocaine-seeking behavior is coincident with phasic dopamine release in the accumbens core and shell. Eur J Neurosci 30: 1117-1127

Paladini CA, Roeper J (2014) Generating bursts (and pauses) in the dopamine midbrain neurons. Neuroscience 282C: 109-121

Panksepp J, Burgdorf J (2000) 50-kHz chirping (laughter?) in response to conditioned and unconditioned tickle-induced reward in rats: effects of social housing and genetic variables. Behav Brain Res 115: 25-38

Panksepp J, Burgdorf J (2003) "Laughing" rats and the evolutionary antecedents of human joy? Physiol Behav 79: 533-547

Parikh V, Pomerleau F, Huettl P, Gerhardt GA, Sarter M, Bruno JP (2004) Rapid assessment of in vivo cholinergic transmission by amperometric detection of changes in extracellular choline levels. Eur J Neurosci 20: 1545-1554

Park J, Aragona BJ, Kile BM, Carelli RM, Wightman RM (2010) In vivo voltammetric monitoring of catecholamine release in subterritories of the nucleus accumbens shell. Neuroscience 169: 132-142

Park J, Bucher ES, Fontillas K, Owesson-White C, Ariansen JL, Carelli RM, Wightman RM (2013) Opposing catecholamine changes in the bed nucleus of the stria terminalis during intracranial self-stimulation and its extinction. Biol Psychiatry 74: 69-76

Park J, Takmakov P, Wightman RM (2011) In vivo comparison of norepinephrine and dopamine release in rat brain by simultaneous measurements with fast-scan cyclic voltammetry. J Neurochem 119: 932-944

Park J, Wheeler RA, Fontillas K, Keithley RB, Carelli RM, Wightman RM (2012) Catecholamines in the bed nucleus of the stria terminalis reciprocally respond to reward and aversion. Biol Psychiatry 71: 327-334

Parsana AJ, Moran EE, Brown TH (2012) Rats learn to freeze to 22-kHz ultrasonic vocalizations through autoconditioning. Behav Brain Res 232: 395-399

Parsons S (2000) Advantages and disadvantages of techniques for transforming and analyzing Chiropteran echolocation calls. J Mammal 81: 927-938

Patel S, Freedman S, Chapman KL, Emms F, Fletcher AE, Knowles M, Marwood R, Mcallister G, Myers J, Curtis N, Kulagowski JJ, Leeson PD, Ridgill M, Graham M, Matheson S, Rathbone D, Watt AP, Bristow LJ, Rupniak NM, Baskin E, Lynch JJ, Ragan CI (1997) Biological profile of L-745,870, a selective antagonist with high affinity for the dopamine D4 receptor. J Pharmacol Exp Ther 283: 636-647

Pearlstein E (2013) Role of descending aminergic pathways in the development of locomotion. Curr Pharm Des 19: 4332-4340

Pereira M, Andreatini R, Schwarting RK, Brenes JC (2014) Amphetamine-induced appetitive 50-kHz calls in rats: a marker of affect in mania? Psychopharmacology (Berl) 231: 2567-77

Peters JL, Yang H, Michael AC (2000) Quantitative aspects of brain microdialysis. Analytic Chimica Acta 412: 1-12

Peterson GB (2004) A day of great illumination: B.F. Skinner's discovery of shaping. J Exp Anal Behav 82: 317-328

Phillips ML, Chase HW, Sheline YI, Etkin A, Almeida JR, Deckersbach T, Trivedi MH (2015) Identifying predictors, moderators, and mediators of antidepressant response in major depressive disorder: neuroimaging approaches. Am J Psychiatry 172: 124-138

Phillips PE, Budygin EA, Robinson DL, Wightman RM (2002) Fast-scan cyclic voltammetry in freely-moving rats. Catecholamine Research: From Molecular Insights to Clinical Medicine. Kluwer Academic/Plenum Publishers, pp 305-313

Picciotto MR, Higley MJ, Mineur YS (2012) Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron 76: 116-129

Planeta CS (2013) Animal models of alcohol and drug dependence. Rev Bras Psiquiatr 35 Suppl 2: S140-S146

Polikov VS, Tresco PA, Reichert WM (2005) Response of brain tissue to chronically implanted neural electrodes. J Neurosci Methods 148: 1-18

Popik P, Kos T, Pluta H, Nikiforuk A, Rojek K, Rygula R (2014) Inhibition of the glucocorticoid synthesis reverses stress-induced decrease in rat's 50-kHz ultrasonic vocalizations. Behav Brain Res 260: 53-57

Porter-Stransky KA, Seiler JL, Day JJ, Aragona BJ (2013) Development of behavioral preferences for the optimal choice following unexpected reward omission is mediated by a reduction of D2-like receptor tone in the nucleus accumbens. Eur J Neurosci 38: 2572-88

Portfors CV (2007) Types and functions of ultrasonic vocalizations in laboratory rats and mice. J Am Assoc Lab Anim Sci 46: 28-34

Pouget JG, Muller DJ (2014) Pharmacogenetics of antipsychotic treatment in schizophrenia. Methods Mol Biol 1175: 557-587

Pritchard LM, Newman AH, McNamara RK, Logue AD, Taylor B, Welge JA, Xu M, Zhang J, Richtand NM (2007) The dopamine D3 receptor antagonist NGB 2904 increases spontaneous and amphetamine-stimulated locomotion. Pharmacol Biochem Behav 86: 718-726

Pugsley TA, Davis MD, Akunne HC, MacKenzie RG, Shih YH, Damsma G, Wikstrom H, Whetzel SZ, Georgic LM, Cooke LW (1995) Neurochemical and functional characterization of the preferentially selective dopamine D3 agonist PD 128907. J Pharmacol Exp Ther 275: 1355-1366

Ramsson ES, Covey DP, Daberkow DP, Litherland MT, Juliano SA, Garris PA (2011a) Amphetamine augments action potential-dependent dopaminergic signaling in the striatum in vivo. J Neurochem 117: 937-948

Ramsson ES, Howard CD, Covey DP, Garris PA (2011b) High doses of amphetamine augment, rather than disrupt, exocytotic dopamine release in the dorsal and ventral striatum of the anesthetized rat. J Neurochem 119: 1162-1172

Ranaldi R (2014) Dopamine and reward seeking: the role of ventral tegmental area. Rev Neurosci 25: 621-630

Redgrave P, Gurney K, Reynolds J (2008) What is reinforced by phasic dopamine signals? Brain Res Rev 58: 322-339

Reed MD, Hildebrand DG, Santangelo G, Moffa A, Pira AS, Rycyna L, Radic M, Price K, Archbold J, McConnell K, Girard L, Morin K, Tang A, Febo M, Stellar JR (2015) Assessing contributions of nucleus accumbens shell subregions to reward-seeking behavior. Drug Alcohol Depend doi: 10.1016/j.drugalcdep.2015.05.001

Reno JM, Marker B, Cormack LK, Schallert T, Duvauchelle CL (2013) Automating ultrasonic vocalization analyses: the WAAVES program. J Neurosci Methods 219: 155-161

Richard JM, Castro DC, Difeliceantonio AG, Robinson MJ, Berridge KC (2013) Mapping brain circuits of reward and motivation: in the footsteps of Ann Kelley. Neurosci Biobehav Rev 37: 1919-1931

Richfield EK, Penney JB, Young AB (1989) Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. Neuroscience 30: 767-777

Robinson DL, Wightman RM (2007) Rapid Dopamine Release in Freely Moving Rats. Ch. 2 in: Electrochemical Methods for Neuroscience (FL): CRC Press.

Roger-Sanchez C, Aguilar MA, Manzanedo C, Minarro J, Rodriguez-Arias M (2013) Neurochemical substrates of MDMA reward: effects of the inhibition of serotonin reuptake on the acquisition and reinstatement of MDMA-induced CPP. Curr Pharm Des 19: 7050-7064

Roitman MF, Stuber GD, Phillips PE, Wightman RM, Carelli RM (2004) Dopamine operates as a subsecond modulator of food seeking. J Neurosci 24: 1265-1271

Rossetti ZL, Pani L, Portas C, Gessa G (1989) Brain dialysis provides evidence for D2-dopamine receptors modulating noradrenaline release in the rat frontal cortex. Eur J Pharmacol 163: 393-395

Rothermel M, Brunert D, Zabawa C, az-Quesada M, Wachowiak M (2013) Transgene expression in target-defined neuron populations mediated by retrograde infection with adeno-associated viral vectors. J Neurosci 33: 15195-15206

Ruskin DN, Rawji SS, Walters JR (1998) Effects of full D1 dopamine receptor agonists on firing rates in the globus pallidus and substantia nigra pars compacta in vivo: tests for D1 receptor selectivity and comparisons to the partial agonist SKF 38393. J Pharmacol Exp Ther 286: 272-281

Rygula R, Pluta H, Popik P (2012) Laughing rats are optimistic. PLoS One 7: e51959

Sadananda M, Natusch C, Karrenbauer B, Schwarting RK (2012) 50-kHz calls in rats: effects of MDMA and the 5-HT(1A) receptor agonist 8-OH-DPAT. Pharmacol Biochem Behav 101: 258-264

Sadananda M, Wohr M, Schwarting RK (2008) Playback of 22-kHz and 50-kHz ultrasonic vocalizations induces differential c-fos expression in rat brain. Neurosci Lett 435: 17-23

Saddoris MP, Sugam JA, Stuber GD, Witten IB, Deisseroth K, Carelli RM (2015) Mesolimbic dopamine dynamically tracks, and is causally linked to, discrete aspects of value-based decision making. Biol Psychiatry 77: 903-911

Sahakian BJ, Robbins TW, Morgan MJ, Iversen SD (1975) The effects of psychomotor stimulants on stereotypy and locomotor activity in socially-deprived and control rats. Brain Res 84: 195-205

Salamone JD, Correa M (2002) Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. Behav Brain Res 137: 3-25

Salamone JD, Correa M (2012) The mysterious motivational functions of mesolimbic dopamine. Neuron 76: 470-485

Salamone JD, Correa M, Farrar A, Mingote SM (2007) Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. Psychopharmacology (Berl) 191: 461-482

Sales GD (1972a) Ultrasound and aggressive behaviour in rats and other small mammals. Anim Behav 20: 88-100

Sales GD (1972b) Ultrasound and mating behaviour in rodents with some observations on other behavioural situations. J Zool 149-164

Sales GD, Pye D (1974) Ultrasonic Communication by Animals. Springer (Nertherlands)

Salmi P (1998) Independent roles of dopamine D1 and D2/3 receptors in rat thermoregulation. Brain Res 781: 188-193

Salmi P, Ahlenius S (2000) Sedative effects of the dopamine D1 receptor agonist A 68930 on rat open-field behavior. Neuroreport 11: 1269-1272

Sanchez-Catalan MJ, Kaufling J, Georges F, Veinante P, Barrot M (2014) The antero-posterior heterogeneity of the ventral tegmental area. Neuroscience 282C: 198-216

Sarter M, Parikh V, Howe WM (2009) Phasic acetylcholine release and the volume transmission hypothesis: time to move on. Nat Rev Neurosci 10: 383-390

Scardochio T, Clarke PB (2013) Inhibition of 50-kHz ultrasonic vocalizations by dopamine receptor subtype-selective agonists and antagonists in adult rats. Psychopharmacology (Berl) 226: 589-600

Schaub CL, Schmelzeis MC, Mittleman G (1997) The effects of limbic lesions on locomotion and stereotypy elicited by dopamine agonists in the rat. Behav Brain Res 84: 129-143

Schindler CW, Carmona GN (2002) Effects of dopamine agonists and antagonists on locomotor activity in male and female rats. Pharmacol Biochem Behav 72: 857-863

Schultz W (1997) Dopamine neurons and their role in reward mechanisms. Curr Opin Neurobiol 7: 191-197

Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80: 1-27

Schultz W (2007a) Behavioral dopamine signals. Trends Neurosci 30: 203-210

Schultz W (2007b) Multiple dopamine functions at different time courses. Annu Rev Neurosci 30: 259-288

Schultz W (2013) Updating dopamine reward signals. Curr Opin Neurobiol 23: 229-238

Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275: 1593-1599

Schwarting RK, Jegan N, Wohr M (2007) Situational factors, conditions and individual variables which can determine ultrasonic vocalizations in male adult Wistar rats. Behav Brain Res 182: 208-222

Seffer D, Schwarting RK, Wohr M (2014) Pro-social ultrasonic communication in rats: Insights from playback studies. J Neurosci Methods 234: 73-81

Segal DS (1975) Behavioral characterization of d- and l-amphetamine: neurochemical implications. Science 190: 475-477

Sellings LH, Clarke PB (2003) Segregation of amphetamine reward and locomotor stimulation between nucleus accumbens medial shell and core. J Neusosci 23: 6295-6303

Sharp T, Zetterstrom T, Ljungberg T, Ungerstedt U (1987) A direct comparison of amphetamine-induced behaviours and regional brain dopamine release in the rat using intracerebral dialysis. Brain Res 401: 322-330

Shin JW, Geerling JC, Loewy AD (2008) Inputs to the ventrolateral bed nucleus of the stria terminalis. J Comp Neurol 511: 628-657

Shnitko TA, Kennerly LC, Spear LP, Robinson DL (2014) Ethanol reduces evoked dopamine release and slows clearance in the rat medial prefrontal cortex. Alcohol Clin Exp Res 38: 2969-2977

Shu Z, Taylor IM, Michael AC (2013) The dopamine patchwork of the rat nucleus accumbens core. Eur J Neurosci 38:3221-9

Simola N, Fenu S, Costa G, Pinna A, Plumitallo A, Morelli M (2012) Pharmacological characterization of 50-kHz ultrasonic vocalizations in rats: Comparison of the effects of different psychoactive drugs and relevance in drug-induced reward. Neuropharmacology 2: 224-234

Simola N, Frau L, Plumitallo A, Morelli M (2013) Direct and long-lasting effects elicited by repeated drug administration on 50-kHz ultrasonic vocalizations are regulated differently: implications for the study of the affective properties of drugs of abuse. Int J Neuropsychopharmacol 17: 429-441

Sirotin YB, Costa ME, Laplagne DA (2014) Rodent ultrasonic vocalizations are bound to active sniffing behavior. Front Behav Neurosci 8: 399

Smith KS, Berridge KC, Aldridge JW (2011) Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. Proc Natl Acad Sci U S A 108: E255-E264

Snoeren EM, Agmo A (2014) The incentive value of males' 50-kHz ultrasonic vocalizations for female rats (Rattus norvegicus). J Comp Psychol 128: 40-55

Soto-Sanchez C, Martinez-Navarrete G, Humphreys L, Puras G, Zarate J, Pedraz JL, Fernandez E (2015) Enduring high-efficiency in vivo transfection of neurons with non-viral magnetoparticles in the rat visual cortex for optogenetic applications. Nanomedicine 11: 835-843

Sparta DR, Stamatakis AM, Phillips JL, Hovelso N, van ZR, Stuber GD (2012) Construction of implantable optical fibers for long-term optogenetic manipulation of neural circuits. Nat Protoc 7: 12-23

Spina L, Fenu S, Longoni R, Rivas E, Di Chiara G (2006) Nicotine-conditioned single-trial place preference: selective role of nucleus accumbens shell dopamine D1 receptors in acquisition. Psychopharmacology (Berl) 184: 447-455

Statistics Canada. CANSIM, Health Indicators. 82-221-X, 105-0501. 2014.

Steinberg EE, Boivin JR, Saunders BT, Witten IB, Deisseroth K, Janak PH (2014) Positive reinforcement mediated by midbrain dopamine neurons requires D1 and D2 receptor activation in the nucleus accumbens. PLoS One 9: e94771

Steinberg EE, Keiflin R, Boivin JR, Witten IB, Deisseroth K, Janak PH (2013) A causal link between prediction errors, dopamine neurons and learning. Nat Neurosci 16: 966-973

Stevenson CW, Goodwin PE, Tunstall B, Spicer CH, Marsden CA, Mason R (2009) Neonatal maternal separation alters reward-related ultrasonic vocalizations in rat dams. Behav Brain Res 200: 232-236

Stuber GD, Hnasko TS, Britt JP, Edwards RH, Bonci A (2010) Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. J Neurosci 30: 8229-8233

Suaud-Chagny MF, Dugast C, Chergui K, Msghina M, Gonon F (1995) Uptake of dopamine released by impulse flow in the rat mesolimbic and striatal systems in vivo. J Neurochem 65: 2603-2611

Sulzer D (2011) How addictive drugs disrupt presynaptic dopamine neurotransmission. Neuron 69: 628-649

Swanson LW (1982) The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 9: 321-353

Takahashi N, Kashino M, Hironaka N (2010) Structure of rat ultrasonic vocalizations and its relevance to behavior. PLoS One 5: e14115

Takmakov P, Zachek MK, Keithley RB, Bucher ES, McCarty GS, Wightman RM (2010) Characterization of local pH changes in brain using fast-scan cyclic voltammetry with carbon microelectrodes. Anal Chem 82: 9892-9900

Taracha E, Hamed A, Krzascik P, Lehner M, Skorzewska A, Plaznik A, Chrapusta SJ (2012) Inter-individual diversity and intra-individual stability of amphetamine-induced sensitization of frequency-modulated 50-kHz vocalization in Sprague-Dawley rats. Psychopharmacology (Berl) 222: 619-632

Thiel KJ, Okun AC, Neisewander JL (2008) Social reward-conditioned place preference: a model revealing an interaction between cocaine and social context rewards in rats. Drug Alcohol Depend 96: 202-212

Thomas DA, Takahashi LK, Barfield RJ (1983) Analysis of ultrasonic vocalizations emitted by intruders during aggressive encounters among rats (Rattus norvegicus). J Comp Psychol 97: 201-206

Thompson B, Leonard KC, Brudzynski SM (2006) Amphetamine-induced 50 kHz calls from rat nucleus accumbens: a quantitative mapping study and acoustic analysis. Behav Brain Res 168: 64-73

Tice MA, Hashemi T, Taylor LA, Duffy RA, McQuade RD (1994) Characterization of the binding of SCH 39166 to the five cloned dopamine receptor subtypes. Pharmacol Biochem Behav 49: 567-571

Trifilieff P, Feng B, Urizar E, Winiger V, Ward RD, Taylor KM, Martinez D, Moore H, Balsam PD, Simpson EH, Javitch JA (2013) Increasing dopamine D2 receptor expression in the adult nucleus accumbens enhances motivation. Mol Psychiatry 18: 1025-1033

Tritsch NX, Ding JB, Sabatini BL (2012) Dopaminergic neurons inhibit striatal output through non-canonical release of GABA. Nature 490: 262-266

Trujillo-Pisanty I, Sanio C, Chaudhri N, Shizgal P (2015) Robust optical fiber patch-cords for in vivo optogenetic experiments in rats. MethodsX 2: 263-271

Tsai HC, Zhang F, Adamantidis A, Stuber GD, Bonci A, de LL, Deisseroth K (2009) Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science 324: 1080-1084

Tzschentke TM (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 56: 613-672

Tzschentke TM (2007) Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol 12: 227-462

van der Poel AM, Noach EJ, Miczek KA (1989) Temporal patterning of ultrasonic distress calls in the adult rat: effects of morphine and benzodiazepines. Psychopharmacology (Berl) 97: 147-148

Vander Weele CM, Porter-Stransky KA, Mabrouk OS, Lovic V, Singer BF, Kennedy RT, Aragona BJ (2014) Rapid dopamine transmission within the nucleus accumbens: dramatic difference between morphine and oxycodone delivery. Eur J Neurosci 40: 3041-3054

Venton BJ, Zhang H, Garris PA, Phillips PE, Sulzer D, Wightman RM (2003) Real-time decoding of dopamine concentration changes in the caudate-putamen during tonic and phasic firing. J Neurochem 87: 1284-1295

Vivian JA, Miczek KA (1991) Ultrasounds during morphine withdrawal in rats. Psychopharmacology (Berl) 104: 187-193

Wachtel SR, Hu XT, Galloway MP, White FJ (1989) D1 dopamine receptor stimulation enables the postsynaptic, but not autoreceptor, effects of D2 dopamine agonists in nigrostriatal and mesoaccumbens dopamine systems. Synapse 4:327-346

Walsh JJ, Han MH (2014) The heterogeneity of ventral tegmental area neurons: Projection functions in a mood-related context. Neuroscience 282C: 101-108

Wanat MJ, Willuhn I, Clark JJ, Phillips PE (2009) Phasic dopamine release in appetitive behaviors and drug addiction. Curr Drug Abuse Rev 2: 195-213

Wassum KM, Ostlund SB, Loewinger GC, Maidment NT (2013) Phasic Mesolimbic Dopamine Release Tracks Reward Seeking During Expression of Pavlovian-to-Instrumental Transfer. Biol Psychiatry 73: 747-755

Wassum KM, Ostlund SB, Maidment NT (2012) Phasic mesolimbic dopamine signaling precedes and predicts performance of a self-initiated action sequence task. Biol Psychiatry 71: 846-854

Wenzel JM, Cheer JF (2014) Endocannabinoid-dependent modulation of phasic dopamine signaling encodes external and internal reward-predictive cues. Front Psychiatry 5: 118

Werb D, Rowell G, Guyatt G, Kerr T, Montaner J, Wood E (2011) Effect of drug law enforcement on drug market violence: a systematic review. Int J Drug Policy 22: 87-94

Wickham RJ, Solecki W, Rathbun LR, Neugebauer NM, Wightman RM, Addy NA (2013) Advances in studying phasic dopamine signaling in brain reward mechanisms. Front Biosci (Elite Ed) 5: 982-999

Widzowski DV, Cory-Slechta DA (1993) Apparent mediation of the stimulus properties of a low dose of quinpirole by dopaminergic autoreceptors. J Pharmacol Exp Ther 266: 526-534

Wightman RM, Amatore C, Engstrom RC, Hale PD, Kristensen EW, Kuhr WG, May LJ (1988) Real-time characterization of dopamine overflow and uptake in the rat striatum. Neuroscience 25: 513-523

Wightman RM, Heien ML, Wassum KM, Sombers LA, Aragona BJ, Khan AS, Ariansen JL, Cheer JF, Phillips PE, Carelli RM (2007) Dopamine release is heterogeneous within microenvironments of the rat nucleus accumbens. Eur J Neurosci 26: 2046-2054

Wightman RM, Robinson DL (2002) Transient changes in mesolimbic dopamine and their association with 'reward'. J Neurochem 82: 721-735

Williams SC, Deisseroth K (2013) Optogenetics. Proc Natl Acad Sci USA 110: 16287

Williams SN, Undieh AS (2010) Brain-derived neurotrophic factor signaling modulates cocaine induction of reward-associated ultrasonic vocalization in rats. J Pharmacol Exp Ther 332: 463-468

Willuhn I, Burgeno LM, Everitt BJ, Phillips PE (2012) Hierarchical recruitment of phasic dopamine signaling in the striatum during the progression of cocaine use. Proc Natl Acad Sci USA 109: 20703-20708

Willuhn I, Tose A, Wanat MJ, Hart AS, Hollon NG, Phillips PE, Schwarting RK, Wohr M (2014) Phasic Dopamine Release in the Nucleus Accumbens in Response to Pro-Social 50 kHz Ultrasonic Vocalizations in Rats. J Neurosci 34: 10616-10623

Willuhn I, Wanat MJ, Clark JJ, Phillips PE (2010) Dopamine signaling in the nucleus accumbens of animals self-administering drugs of abuse. Curr Top Behav Neurosci 3: 29-71

Wintink AJ, Brudzynski SM (2001) The related roles of dopamine and glutamate in the initiation of 50-kHz ultrasonic calls in adult rats. Pharmacol Biochem Behav 70: 317-323

Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5: 483-494

Witten IB, Steinberg EE, Lee SY, Davidson TJ, Zalocusky KA, Brodsky M, Yizhar O, Cho SL, Gong S, Ramakrishnan C, Stuber GD, Tye KM, Janak PH, Deisseroth K (2011) Recombinase-driver rat lines: tools, techniques, and optogenetic application to dopamine-mediated reinforcement. Neuron 72: 721-733

Wohr M, Houx B, Schwarting RK, Spruijt B (2008) Effects of experience and context on 50-kHz vocalizations in rats. Physiol Behav 93: 766-776

Wohr M, Rippberger H, Schwarting RK, van Gaalen MM (2015) Critical involvement of 5-HT2C receptor function in amphetamine-induced 50-kHz ultrasonic vocalizations in rats. Psychopharmacology (Berl) 232: 1817-1829

Wohr M, Schwarting RK (2007) Ultrasonic communication in rats: can playback of 50-kHz calls induce approach behavior? PLoS One 2: e1365

Wöhr M, Schwarting RK (2012) Rodent ultrasonic communications and its relevance for models of neuropsychiatric disorders. E-Neuroforum 1: 71-80

Wohr M, Schwarting RK (2013) Affective communication in rodents: ultrasonic vocalizations as a tool for research on emotion and motivation. Cell Tissue Res 354: 81-97

Wright CI, Groenewegen HJ (1995) Patterns of convergence and segregation in the medial nucleus accumbens of the rat: relationships of prefrontal cortical, midline thalamic, and basal amygdaloid afferents. J Comp Neurol 361: 383-403

Wright JM, Deng L, Clarke PB (2012a) Failure of rewarding and locomotor stimulant doses of morphine to promote adult rat 50-kHz ultrasonic vocalizations. Psychopharmacology (Berl) 224: 477-487

Wright JM, Dobosiewicz MR, Clarke PB (2012b) alpha- and beta-Adrenergic receptors differentially modulate the emission of spontaneous and amphetamine-induced 50-kHz ultrasonic vocalizations in adult rats. Neuropsychopharmacology 37: 808-821

Wright JM, Dobosiewicz MR, Clarke PB (2012c) The role of dopaminergic transmission through D1-like and D2-like receptors in amphetamine-induced rat ultrasonic vocalizations. Psychopharmacology (Berl) 225: 853-868

Wright JM, Gourdon JC, Clarke PB (2010) Identification of multiple call categories within the rich repertoire of adult rat 50-kHz ultrasonic vocalizations: effects of amphetamine and social context. Psychopharmacology (Berl) 211: 1-13

Xi ZX, Gardner EL (2007) Pharmacological actions of NGB 2904, a selective dopamine D3 receptor antagonist, in animal models of drug addiction. CNS Drug Rev 13: 240-259

Yee N, Schwarting RK, Fuchs E, Wohr M (2012) Juvenile stress potentiates aversive 22-kHz ultrasonic vocalizations and freezing during auditory fear conditioning in adult male rats. Stress 15: 533-544

Yuan J, Chen X, Brodbeck R, Primus R, Braun J, Wasley JW, Thurkauf A (1998) NGB 2904 and NGB 2849: two highly selective dopamine D3 receptor antagonists. Bioorg Med Chem Lett 8: 2715-2718

Yuki S, Okanoya K (2014) Behavioral Correlates of 50-kHz Ultrasonic Vocalizations in Rats: Progressive Operant Discrimination Learning Reduces Frequency Modulation and Increases Overall Amplitude. Animal Behavior and Cognition 1: 452-463

Zahm DS (1999) Functional-anatomical implications of the nucleus accumbens core and shell subterritories. Ann N Y Acad Sci 877: 113-128

Zeskind PS, McMurray MS, Cox Lippard ET, Grewen KM, Garber KA, Johns JM (2014) Translational analysis of effects of prenatal cocaine exposure on human infant cries and rat pup ultrasonic vocalizations. PLoS One 9: e110349

Zhang F, Gradinaru V, Adamantidis AR, Durand R, Airan RD, de LL, Deisseroth K (2010) Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. Nat Protoc 5: 439-456

Zhang-James Y, Yang L, Middleton FA, Yang L, Patak J, Faraone SV (2014) Autism-related behavioral phenotypes in an inbred rat substrain. Behav Brain Res 269: 103-114

APPENDICES

Appendix A: Supplementary Material for Chapter 2

Appendix B: Supplementary Material for Chapter 3

Appendix C: Supplementary Material for Chapter 4

Appendix D: Copyright Waivers and Permissions

Appendix E: Copy of Published Manuscript

Appendix A: Supplementary Material for Chapter 2

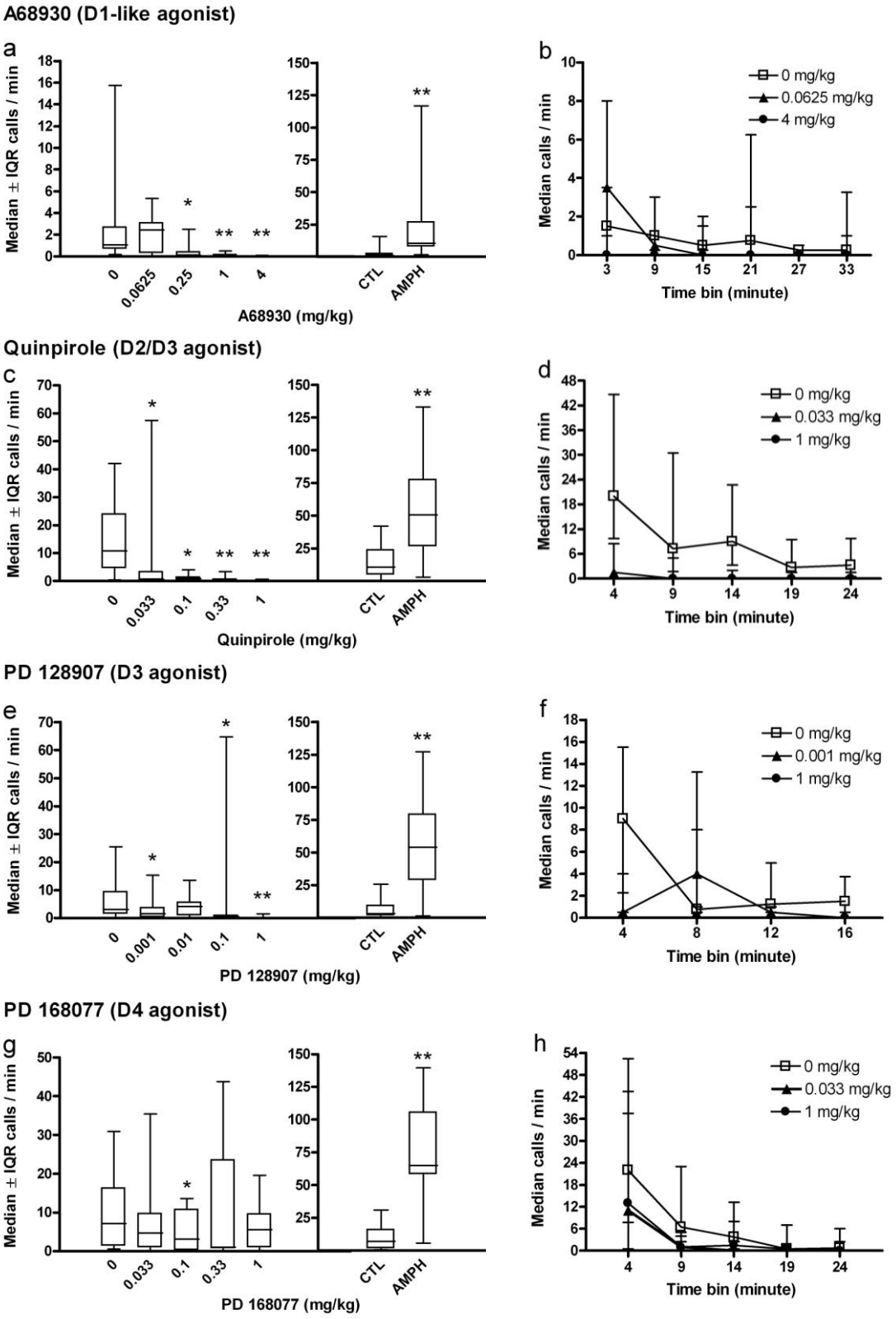

Fig. S1 Experiments 1, 2, 3 and 4: Dose-dependent (**a**, **c**, **e** and **g**) and time-dependent (**b**, **d**, **f** and **h**) effects of DAergic agonists on 50-kHz call rate. Panels **a**, **c**, **e** and **g** are box plots showing median ± IQR (interquartile range). The lowest and highest doses in these left-hand panels are represented as median (± IQR) calls per 1-min time bin in panels **b**, **d**, **f** and **h**, respectively. The highest dose of each agonist, except the D4 agonist, virtually abolished calls even at the earliest time point (**b**, **d**, **f** and **h**). Each agonist was tested on a different group of rats (n=12), and each rat was tested at all doses of a given agonist and with amphetamine 1 mg/kg IP (AMPH). *p<0.05, **p<0.01 *vs*. zero dose (Wilcoxon's tests). The same vehicle condition is shown twice in panels **a**, **c**, **e** and **g** (i.e. 0 and CTL).

Appendix B: Supplementary Material Chapter for Chapter 3

Table S1: Summary of drugs used for the pharmacological verification of dopaminergic release

Treatment						
Drug		**Dose (mg/kg)**	**Route**	**Time before testing (min)**	**Anticipated effect on DA current**	**n (rats)**
D1/D2 agonist	quinpirole	0, 0.3	IP	20	Decrease	1
NET blocker + α2-adrenoreceptor antagonist	DMI + yohimbine	0, 15 + 0, 6		20	No change	1
α2-adrenoreceptor antagonist	yohimbine	0, 6		20	No change (or small increase)	1
DAT blocker	GBR12909	0, 15		30	Increase	3
D2/D3 antagonist	raclopride	0, 2		30	Increase	2
Indirect DA/NA agonist	amphetamine	0, 1.6		--	Increase	3

Table S2 Completed drug tests for each rat, used for the pharmacological validation of *in vivo* dopamine signals

Rat ID	SAL	DMSO	AMPH	GBR	QUIN	RAC	DMI/YO	YO
8	✓	✓	✓	✓	✓	✓		✓
10	✓	✓	✓	✓		✓	✓	
11	✓	✓	✓	✓				

SAL (saline), DMSO (dimethyl sulfoxide), AMPH (amphetamine), GBR (GBR12909), QUIN (quinpirole), DMI (desipramine), YO (yohimbine), RAC (raclopride)

Table S3 Optimized stimulation parameters identified for each rat following optical self-stimulation training

	Frequency	
Rat ID	# Pulses	Period (ms)
BeChR20	57	17.86
BeChR25	51	19.90
BeChR28	28	35.70
BeChR29	34	29.41
BeChR30	59	16.95

Table S4 Summary of MFB stimulation parameters tested for each rat with DA transient and USV emission results

	Stimulation parameters																					
	30Hz										60Hz											
	40µA		60µA		80µA		100µA		120µA		40µA		60µA		80µA		100µA		120µA		140µA	
Rat	DA	USV	DA	USV	DA	USV	DA	USV	DA	USV	DA	USV	DA	USV	DA	USV	DA	USV	DA	USV	DA	USV
8																						*
10																						
11																						

* 50- and 22-kHz calls

Note: each cell represents one stimulation trial

Not tested | DA and USVs absent | DA and USVs present | Only DA present

Table S5 Lever press data, broken down by rat and schedule. The number of lever presses is shown for the entire 2-hour session and for the first 30 minutes. REIN= reinforced and UNREIN= unreinforced lever press.

Rat	FI1				FI2				VI1				VI2			
	120 min		30 min		120 min		30 min		120 min		30 min		120 min		30 min	
	UNREIN	REIN	UNREIN	REIN	UNREIN	REIN	UNREIN	REIN	UNREIN	REIN	UNREIN	REIN	UNREIN	REIN	UNREIN	REIN
20	1033	308	182	70	965	328	177	76	1253	279	296	72	807	283	197	75
25	440	291	114	65	488	312	107	72	503	197	139	83	233	203	71	54
29	2018	331	423	78	2362	340	395	80	1367	263	242	64	2033	515	219	107
30	747	328	104	79	1133	333	171	75	1598	280	287	68	1518	300	236	71

Table S6 Call percentages (14 subtypes and 2 categories) before and after optogenetic stimulation of the VTA, under four reinforcement schedules

SCHEDULE	TIMING	RAT	T	F	FT	CX	MS	C	UR	DR	SU	SD	TJ	SP	S	IU	M	UN
FI	Before	**20**	43	17	7	15	4	0	0	4	2	0	4	0	2	2	0	0
	After		41	23	2	14	0	0	0	7	0	0	7	0	0	2	0	4
FT	Before		20	34	10	8	2	0	0	0	10	3	5	0	4	1	0	3
	After		20	35	9	6	5	0	1	4	6	2	4	1	2	2	1	2
VI	Before		24	28	5	12	6	0	0	7	3	1	5	1	5	2	0	1
	After		35	28	7	11	9	0	0	2	0	0	1	0	5	0	0	2
VT	Before		19	40	12	6	3	1	0	3	4	2	4	0	2	3	0	1
	After		26	47	3	3	2	0	0	3	3	2	5	2	2	0	0	2
FI	Before	**25**	43	12	20	3	5	4	0	0	5	2	2	0	2	1	0	1
	After		47	12	20	2	4	3	1	0	5	2	1	0	2	0	0	1
FT	Before		55	7	19	3	2	2	1	0	4	1	3	0	1	1	0	1
	After		52	7	21	3	2	3	0	0	5	1	3	0	1	1	0	1
VI	Before		56	4	15	7	3	2	0	0	3	3	3	0	2	1	0	1
	After		47	11	18	5	5	0	0	0	4	4	3	0	2	1	0	0
VT	Before		49	7	14	2	2	6	1	0	2	2	13	0	1	1	0	0
	After		53	7	13	2	1	6	1	1	2	0	14	0	0	0	0	0
FI	Before	**29**	20	44	8	4	8	0	0	4	4	0	0	0	4	4	0	0
	After		23	18	5	18	0	0	0	0	9	9	0	5	8	5	0	0
FT	Before		58	9	8	10	1	1	0	1	5	1	0	0	3	2	0	1
	After		49	8	11	7	1	0	0	1	7	1	0	0	7	6	0	2
VI	Before		47	10	7	7	0	3	0	3	10	0	0	0	10	3	0	0
	After		44	6	0	25	0	0	0	0	6	0	0	0	19	0	0	0
VT	Before		44	16	10	8	1	1	1	0	8	2	4	0	3	2	0	0
	After		42	20	10	9	2	1	0	0	5	3	3	0	4	0	0	1
FI	Before	**30**	66	8	6	2	0	1	1	1	1	0	3	0	7	0	0	4
	After		71	5	4	2	1	1	0	1	1	2	1	0	9	0	0	2
FT	Before		80	1	10	1	1	2	0	0	1	0	3	0	1	0	0	0
	After		77	2	12	1	0	2	0	0	2	0	3	0	1	0	0	0
VI	Before		78	2	7	1	0	0	0	0	2	1	5	0	3	0	0	1
	After		77	2	8	2	0	1	0	0	2	1	5	0	1	0	0	1
VT	Before		73	3	11	2	1	1	0	0	2	0	5	0	1	0	0	1
	After		70	2	12	2	2	1	0	0	3	0	7	0	1	0	0	0

T: trill; F: flat; FT: flat-trill; CX: complex; MS: multistep; C: complex; UR: up-ramp; DR: down-ramp; SU: step-up; SD: step-down; TJ: trill-jump; SP: split; S: short; IU: inverted-U; M: miscellaneous; UN: unclassifiable (as described in Wright et al. 2010)

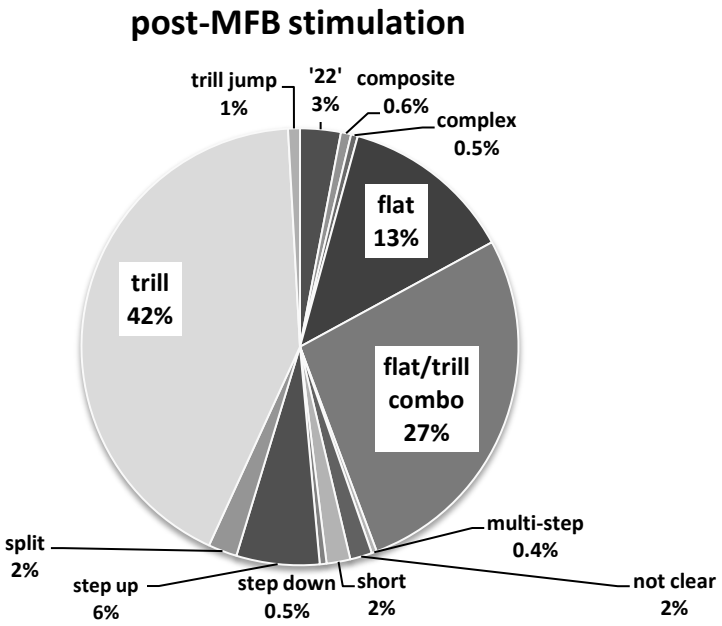

Fig. S1 Call profile following electrical stimulation of the MFB (n=3 rats). Each sector represents the group mean number of calls in a given subtype, expressed as a percentage.

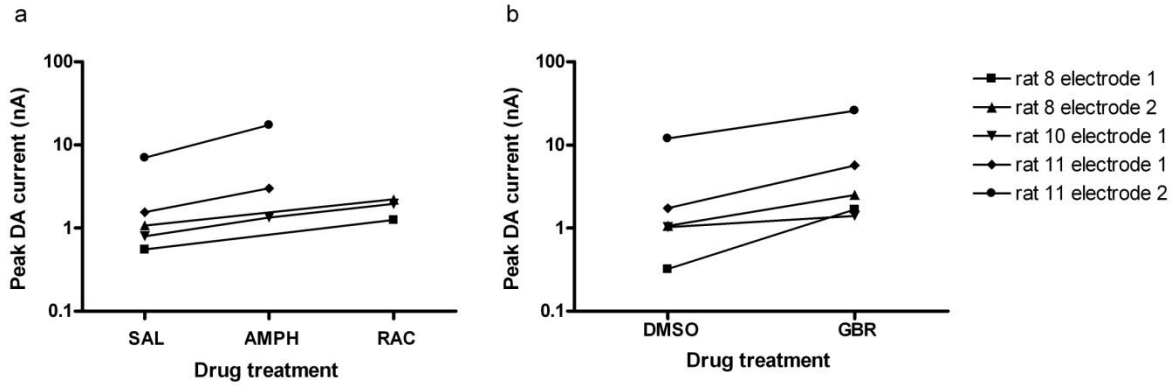

Fig. S2 Pharmacological validation of dopaminergic release from voltammetric recordings in the nucleus accumbens. Panel **a** shows a significant increase in peak DA current following an acute administration of the indirect DA/NA agonist amphetamine (AMPH) (paired t-test, $p<0.05$) and the D2 antagonist raclopride (RAC) (paired t-test, $p<0.05$). Panel **b** show a significant increase in

peak DA current following an acute injection of the DAT blocker GBR12909 (GBR) (paired t-test, $p<0.02$). Vehicle conditions: SAL (saline), DMSO (dimethyl sulfoxide).

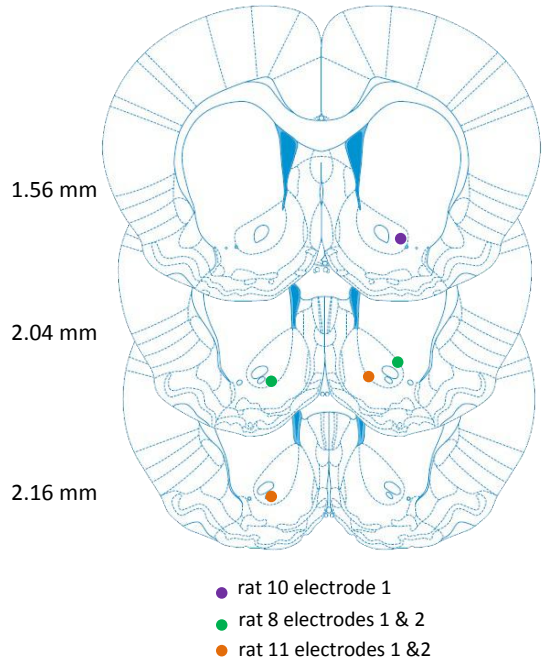

Fig. S3 Location of voltammetric microsensors (n=4, two microsensors per rat). Each point represents the approximate center of a microsensor. Coordinates are in millimeters anterior to bregma and the coronal drawings are from the Paxinos and Watson (2006) atlas.

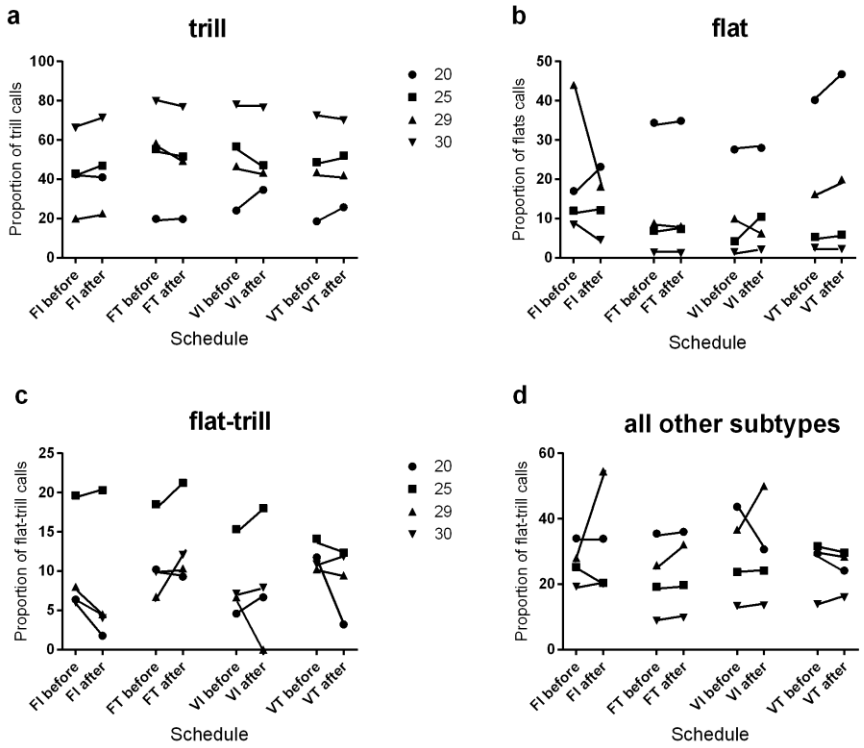

Fig. S4 Proportions of 50-kHz call subtypes for each rat and for each reinforcement schedule, before and after optogenetic stimulation of VTA DAergic neurons. Panels **a**, **b** and **c** show the proportions of trill, flat and flat-trill call subtypes, i.e. the three most common calls observed. Panel **d** is the total proportion of other calls pooled together (13 subtypes). FI: fixed interval, FT: fixed time, VI: variable interval, VT: variable time

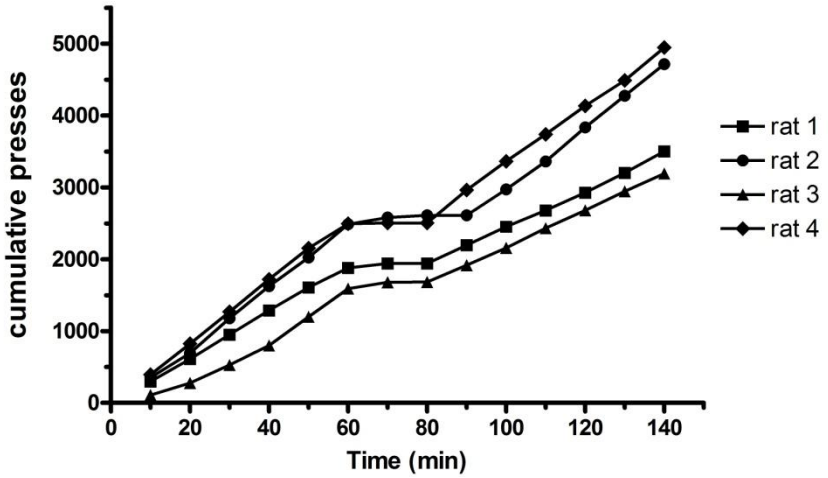

Fig. S5 Cumulative lever presses during optogenetic self-stimulation of VTA DAergic neurons. Rats (n=4) were trained to hold down a lever for 2 s (which could be accumulated across different hold-downs) to obtain a 1 s train of 5 ms light pulses into the VTA (28-56 Hz). A 2 s blackout period (lever retraction) followed each reward. The laser for optogenetic stimulation was turned on during the first 6 sessions (time 10-60), was turned off for the two next sessions (minutes 60-80) and subsequently turned back on. Rats only pressed when stimulation was available.

Appendix C: Supplementary Material for Chapter 4

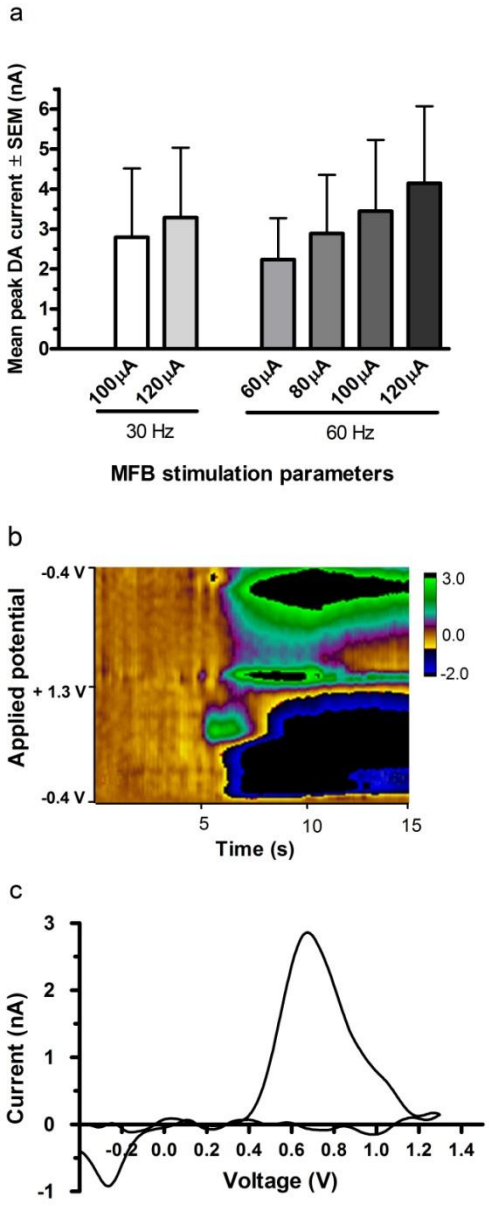

Fig. S1 Phasic DA release in the NAcc occurs immediately following electrical stimulation of the MFB (60 Hz, 24 pulses, biphasic, 2ms/phase, 100µA). Panel **a** shows mean peak DA currents across various stimulation parameters. Panel **b** is a color plot from a representative rat, showing changes in dopamine current (color) in relation to applied potential (y-axis) and time (x axis), with onset of MFB stimulation occurring at 5s. The unfiltered data were subjected to a single two-dimensional smooth (a 5 point running average in both X and Y dimensions). Panel **c** shows

the background-subtracted cyclic voltammogram from the same rat at the time of the peak DA current seen in panel **b**, with the peak current occurring at the expected oxidation potential of dopamine, i.e. approximately 0.65V (vs. Ag/AgCl reference).

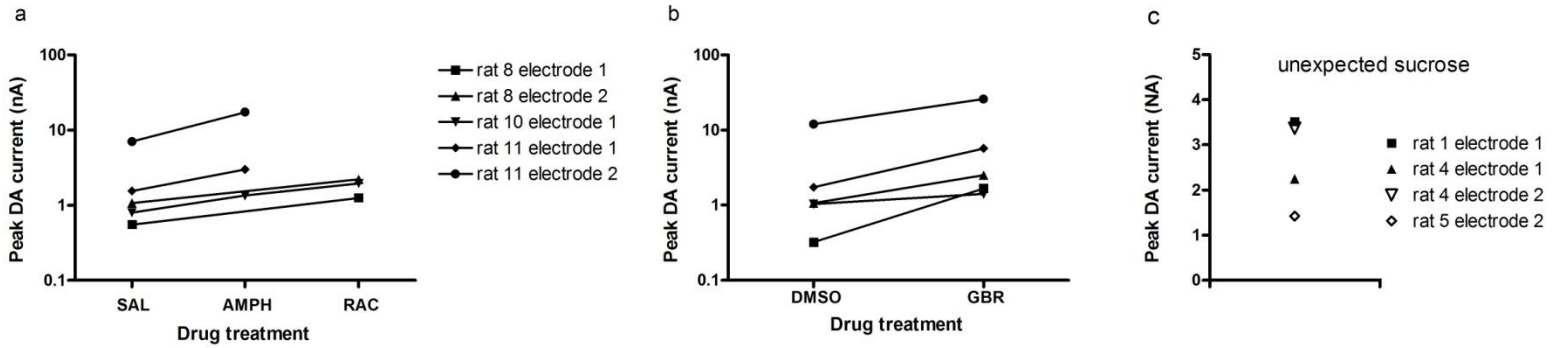

Fig. S2 Validation of voltammetric signals in the nucleus accumbens, using pharmacological treatments and a behaviourally salient stimulus (unexpected sucrose pellet). Each line represents one electrode in panels a and b. Panel **a** shows a significant increase in peak DA current following an acute administration of the indirect DA/NA agonist amphetamine (AMPH) (paired t-test, $p<0.05$) and the D2 antagonist raclopride (RAC) (paired t-test, $p<0.05$). Panel **b** shows a significant increase in peak DA current following an acute injection of the DAT blocker GBR12909 (GBR) (paired t-test, $p<0.02$). Panel c shows the measured DA current for each electrode following unexpected sucrose delivery. Vehicle conditions: SAL (saline), DMSO (dimethyl sulfoxide). Panel **c** shows the peak current of the DA transient following unexpected sucrose delivery.

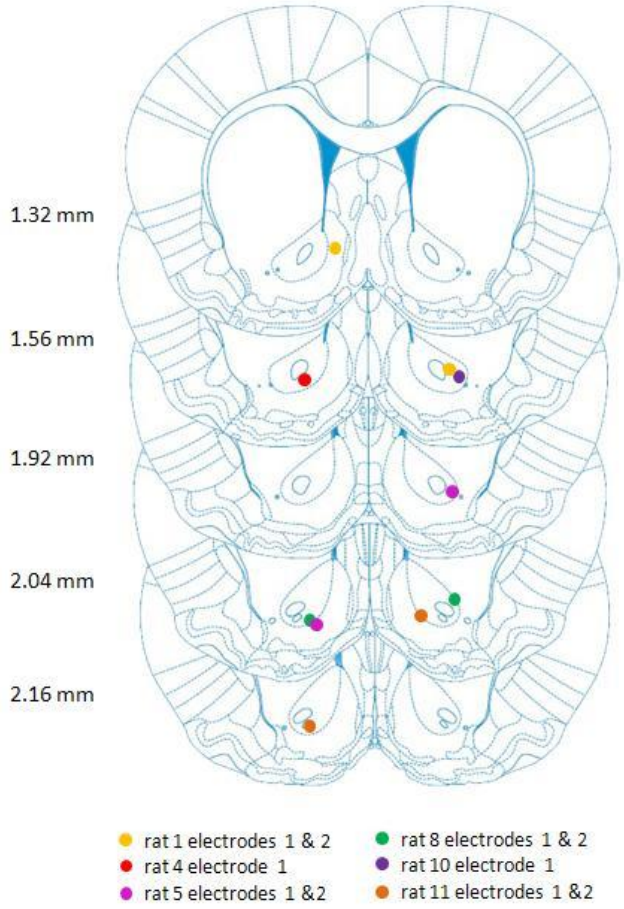

Fig. S3 Location of voltammetric electrodes (n=10 electrodes). Each point represents the approximate center of a microsensor. Coordinates are in millimeters anterior to bregma and the coronal drawings are taken from the Paxinos and Watson (2006) rat brain atlas.

Table S1 Completed drug tests for each rat, used for the pharmacological validation of electrical-induced *in vivo* dopamine signals

Rat ID	SAL	DMSO	AMPH	GBR	QUIN	RAC	DMI/YO	YO
8	✓	✓	✓	✓	✓	✓		✓
10	✓	✓	✓	✓		✓	✓	
11	✓	✓	✓	✓				

SAL (saline), DMSO (), AMPH (amphetamine), GBR (GBR12909), QUIN (quinpirole), DMI (desipramine), YO (yohimbine), RAC (raclopride)

Table S2 Summary of drugs used for the pharmacological verification of dopaminergic release

Treatment					
Drug		**Dose (mg/kg)**	**Route**	**Time before testing (min)**	**Anticipated effect on DA current**
D1/D2 agonist	quinpirole	0, 0.3	IP	20	Decrease
NET blocker + α2-adrenoreceptor antagonist	DMI + yohimbine	0, 15 + 0, 6		20	No change
α2-adrenoreceptor antagonist	yohimbine	0, 6		20	No change (or small increase)
DAT blocker	GBR12909	0, 15		30	Increase
D2/D3 antagonist	raclopride	0, 2		30	Increase
Indirect DA/NA agonist	amphetamine	0, 1.6		(immediately)	Increase

Table S3 Completed verifications for the characterization of *in vivo* dopamine signals

Rat ID	Stimulated release	Unexpected sucrose	Pharmacological validation	Histology/lesion
1		✓		✓
4		✓		✓
5		✓		✓
8	✓		✓	✓
10	✓		✓	✓
11	✓		✓	✓