CHAPTER SEVEN

Pharmacological Animal Models of Tic Disorders

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Abstract

This review summarizes animal models of Tourette syndrome (TS) and associated tic disorders that have been developed through pharmacological manipulation. These models provide a useful platform to explore the pathophysiology and the therapeutic interventions available for these disorders. The current pharmacological models, primarily using rodents and nonhuman primates, are classified in this review into two major categories depending on the methodology used for administration, that is, systemic and focal (intracerebral) injection protocols. The systemic protocol primarily targets monoamines such as dopamine and serotonin, whereas the focal protocol mainly manipulates local transmission of gamma-aminobutyric acid (GABA). Each category is capable of inducing behavioral abnormalities that are characteristic of TS spectrum disorders, ranging from sensorimotor to cognitive and emotional symptoms to various degrees. Among a variety of pharmacological models, focal microinjection of GABA antagonists into the sensorimotor striatum has helped identify abnormal neural discharge in the global networks which underlie tourettism, including not only the cerebral cortex and basal ganglia but also the cerebellum, consistent with recent neuroimaging studies for TS subjects. This unique model also provides the opportunity to clarify the effect and mechanisms of therapeutic deep brain stimulation. Continuing efforts to incorporate cutting-edge knowledge into the existing models, as well as to combine different model platforms, will allow further refinement of animal models, thereby leading to a greater understanding of TS and associated tic disorders.
1. INTRODUCTION

The development of animal models of Tourette syndrome (TS) has been a continuing direction in medical research. Yet due to the complexity of its etiology and phenotype, the disorder has proved to be a difficult condition to model. In fact, there is no single instance that captures all the underlying pathologies and symptom profiles. Nevertheless, various experimental platforms, primarily using rodents and nonhuman primates, have been reported in the literature, each successfully mimicking some aspects of the disorder through different experimental manipulations. The purpose of this review is to summarize recent advances in the development of pharmacological animal models, and to compare their relative strengths and weaknesses with a view to suggesting future refinements and research directions.

The symptoms of TS are wide ranging, including motor, cognitive, and emotional abnormalities. In particular, the involuntary movement varies in form, severity, and the areas of body affected. Tics range from short myoclonic jerks that involve only one or a few muscles to complex motor or vocal tics that involve sequential activation of several muscle groups. The
neuropsychiatric comorbidities typically manifest as obsessive–compulsive disorder (OCD) and attention deficit hyperactivity disorder (ADHD), with patients also at risk of increased incidence of depression and anxiety (de la Tourette, 1885; Obeso, Rothwell, & Marsden, 1982; The Tourette Syndrome Classification Study Group, 1993). Furthermore, the involuntary nature of tic movement could also vary across individuals. It has been shown that the majority of TS patients describe a motor tic as a voluntary motor response to an involuntary sensation (premonitory urge), rather than a completely involuntary movement (Kwak, Dat, & Jankovic, 2003).

The cause of TS has yet to be fully elucidated, but the pathophysiology most likely involves neural circuits linking the cerebral cortex and basal ganglia (BG) (Albin & Mink, 2006; Mink, 2001; Peterson, 2001; Sowell et al., 2008; Worbe et al., 2010). Recent advances in functional neuroanatomy have made it possible to hypothesize that dysfunction in specific cerebro–BG circuits leads to specific clinical manifestations. It has been shown that cerebral regions associated with sensorimotor, associative (i.e., cognitive), and limbic (i.e., emotional) functions connect with the striatum in a topographically organized manner. Specifically, sensorimotor input projects to the dorsolateral division, limbic input to the ventromedial division, and associative input to in between the two divisions (Alexander, DeLong, & Strick, 1986; Francois et al., 2004; Haber, 2003; Hoover & Strick, 1993; Parent & Hazrati, 1995; Percheron & Filion, 1991). Such region-specific symptom expressions are indeed demonstrated in monkeys, in which disruptions in the motor, associative, and limbic division in the BG can cause behavioral changes that share similar features with TS, ADHD, and OCD, respectively (Baup et al., 2008; Francois et al., 2004; Grabli et al., 2004; Rotge et al., 2012; Worbe et al., 2009). These findings may provide the foundation for a perspective that these disorders can individually emerge as a single clinical entity but can also appear as comorbidities of one another depending on the site and extent of abnormalities.

In this review, we first introduce the concept of validity criteria that will be useful for developing animal models in general. We then summarize pharmacological models of tic disorders. These models utilize rodent and nonhuman primate platforms and can be classified into two categories depending on whether pharmacological compounds are delivered systemically or intracranially in a circuit-specific manner. Each category can display a range of behavioral abnormalities that are typical of TS symptomatology, that is, motor, cognitive, and emotional manifestations.
2. VALIDITY CRITERIA FOR DEVELOPING ANIMAL MODELS

Animal models for scientific and medical research are developed for a number of reasons and are predicated on being able to satisfy a multitude of research requirements. Typically, the purpose of creating an animal model is to investigate the mechanisms underlying physiological and pathological states. These investigations can then lead to the development of more sophisticated models of a disease or the assessment of the effects of interventions in a specific disease condition. Therefore, the development of preclinical animal models requires that explicit premises about a specific disease or symptom be established prior to platform creation and its utilization. This initial step is required so that the model has validity with respect to the condition being tested (Massoud et al., 1998; van der Staay, Arndt, & Nordquist, 2009).

The validity of an experimental platform is established scientifically through its ability to generate consistent data. Thus, a valid model must demonstrate a level of reliability such that the same experiments conducted independently in different laboratories produce the same results. In addition, an appropriate model should satisfy the following criteria as much as possible: strong phenomenological similarities (face validity), comparable etiology (construct validity), and common therapeutic responses (predictive validity) (Swerdlow & Sutherland, 2006). It is generally considered that the more criteria (face, construct, and validity) that a model satisfies, the more relevance the model has to the clinical condition being tested, and the robustness of the findings that can be extrapolated from the model.

2.1. Face validity

Face validity is reached when the model in question demonstrates phenomenological similarity in symptom profiles to the clinical condition being investigated. It has been suggested that face validity is the starting point for the development of an animal model, and in fact may be the most important criterion for establishment of a preclinical test platform (Holmes, 2003). However, the reliance on face validity as the sole or primary criterion for an animal model is subject to a major conflicting bias. By emphasizing face validity, it is possible that a valid animal model in a lower phylogenetic animal species will be overlooked (Swerdlow & Sutherland, 2006; van der Staay et al., 2009).
2.2. Predictive validity

Predictive validity means that performance in the test predicts performance in the condition being modeled. By extension, it allows extrapolation of the observed responses to be applied to other species, testing, and clinical environments (Epstein, Preston, Stewart, & Shaham, 2006; van der Staay et al., 2009). An especially important component of predictive validity is its use in screening drugs for therapeutic potential or efficacy. It is often the case that these models utilize behavioral paradigms that have no resemblance to the clinical condition as recognized in human patients. An example includes the use of dopamine (DA) antagonists to prevent apomorphine-induced vomiting in canines as a measure of an antipsychotic potential (Depoortere, Barret-Grevoz, Bardin, & Newman-Tancredi, 2008; Scherkl, Hashem, & Frey, 1990).

Although predictive models are a mainstay of animal test platforms, their use for screening potential therapies may be confounded by the idiosyncratic nature of each species physiology. For example, a therapy may be efficacious in an animal model but not in human subjects, or the model may fail to detect a therapeutic response that would otherwise be useful for the intended disease (Whiteside, Adedoyin, & Leventhal, 2008).

2.3. Construct validity

The concept of construct validity requires that the theoretical basis underlying the model matches pathophysiological observations in patients (Epstein et al., 2006; van der Staay et al., 2009). Construct validity is therefore a theory/etiology-driven substantiation of the physiological and behavioral components of the model (Sarter & Bruno, 2002). In other words, construct validity establishes a relationship between the physiological and behavioral manifestations in the test platform and the hypothesized pathologies in the clinical condition. Therefore, construct validity is achieved when the manipulation targets the specific system which is believed to underlie the symptoms of any disorder. In the case of tic/TS disorders, the current theoretical constructs suggest an abnormality of cerebrostriatal-thalamic circuits, with several causal factors hypothesized, such as immunological complications, genetic/epigenetic changes in early brain development—leading to disorders of synaptic transmission involving monoamine, gamma-aminobutyric acid (GABA)ergic, and glutamatergic networks. Therefore, any model which set out to model tics/TS and targeted any of the suspected causes or neuronal systems would satisfy...
construct validity. A more detailed discussion of the different criteria underlying the concept of validity can be found in the following publications (Swerdlow & Sutherland, 2006; van der Staay, 2006; van der Staay et al., 2009; Willner, 1984).

3. ANIMAL MODELS OF TIC DISORDERS

Due to the wide range of symptoms associated with TS, their classification can be troublesome when developing and utilizing experimental animal models. At first glance, its principal symptom, motor tics, appears to suggest that the condition should be treated as a movement disorder. However, other TS-associated symptoms such as OCD, ADHD, and premonitory urges imply that the disorder should instead be considered a neuropsychiatric abnormality. As a consequence, any of the animal models for TS has some limitations with respect to the validity criteria discussed earlier, for example, a particular model may display excellent myoclonic-type tics, but not any of the more complex neuropsychiatric abnormalities associated with the disorder.

The pharmacological animal models currently available can be categorized into two groups on the basis of the route for the induction of the symptoms: that is, systemic and focal (intracerebral) administrations. In the systemic administration model, manipulation of monoamines such as DA and 5-hydroxytryptamine (5-HT or serotonin) are the two major compounds most frequently targeted through specific agonists/antagonists, although some studies focus on norepinephrine for its effects on sensorimotor gating. In the focal administration model, the most reliable compounds in terms of validity constructs are those that modulate local GABAergic transmission, such as bicuculline, however, monoamine agonists/antagonists can also be used. Whereas systemic administration is superior in terms of ease of use, focal administration has the advantage that the intensity and specificity of the symptoms can be controlled, at least to some extent, by employing site-specific injections within the responsible neural circuits. We now review each type of the model in detail in the following sections, with an emphasis on the focal bicuculline model.

3.1. Systemic administration of pharmacological compounds

A common method for modeling tic disorders is to use systemic administration of pharmacological compounds targeting different neurotransmitter systems via intramuscular injection. From experimental perspectives,
systemic administration has a number of desirable features, such as its ease of use. Moreover, unlike intracerebral drug administration through an injection cannula, the brain tissue is not damaged with the systemic approach. There are several compounds that can induce TS-like behavior when administered systemically. The principal drugs among these are those that modulate monoamines, especially DA and 5-HT.

3.1.1 Dopaminergic models for the tic expression

The hypothesis that DA plays an integral part in TS has been a central tenet of theories to explain the pathophysiology of tic behavior for many years, for review see (Albin, 2006; Albin & Mink, 2006). DA is a monoamine neurotransmitter synthesized in “DA neurons” in the midbrain, which project to and innervate large regions of the brain including the motor and limbic divisions of the striatum and cortical mantle (Anden et al., 1964; Dahlstroem, Fuxe, Olson, & Ungerstedt, 1964). PET imaging has shown abnormalities in DA transmission in TS patients (Singer et al., 2002). Moreover, DA antagonists (e.g., haloperidol—a postsynaptic D2 receptor antagonist) are routinely prescribed to patients with TS and other tic disorders (Ross & Moldofsky, 1978; Seignot, 1961; Shapiro, Shapiro, & Eisenkraft, 1983). It is this successful use of DA antagonists that has provided the main impetus for establishing a role for DA in TS.

With respect to animal models, systemic administration of DA agonists (e.g., amphetamine, cocaine) can induce stereotypic behaviors, which are considered a form of complex motor tic that are analogous to obsessive—compulsive behavior in human behavior. These stereotypies manifest as excessive grooming, biting, and licking and are often species specific (Randrup & Munkvad, 1967; Randrup, Munkvad, & Udsen, 1963), for review see Randrup and Munkvad (1974). Although these methods do not induce myoclonic-like tics that are characteristic of TS, they are important for investigating the neuropsychiatric abnormalities associated with the disorder. In particular, OCD in TS can range from complex sequential behaviors to short simple movements, and the types of behavior expressed after DA agonist administration could potentially provide insights into the neural mechanisms of these behaviors. Recent studies utilizing systemic delivery of DA agonists and antagonists in rodents have shown that stereotypic behavioral profiles depend on whether D1 receptors are activated directly by D1-specific agonists or are upregulated through chronic exposure to D1 antagonists (Taylor, Rajbhandari, Berridge, & Aldridge, 2010). If animals are acutely challenged with D1
agonists, they display “sequential super-stereotypy” and overexpress complete grooming chains. Sudden withdrawal of D1 antagonists after chronic exposure leads to a rapid upregulation of D1 receptors, which in turn leads to the expression of simple stereotypies including intense scratching and biting behaviors.

Another major advantage of these models is that they can be used to investigate the genetic and biochemical changes associated with OCD behaviors. It has been shown that induction of stereotypy by systemic administration of DA agonists is accompanied by specific genetic activation in the striatum and cerebral cortex, in particular upregulation of the *fos–jun* family in striatal striosomes (Canales & Graybiel, 2000a, 2000b; Graybiel, Moratalla, & Robertson, 1990). Interestingly, the ratio of striosomal to matrix early gene expression increases as the severity of the stereotypy increases. It has been shown that striosomes receive inputs from limbic regions of the cortical mantle (e.g., orbitofrontal cortex and anterior cingulate cortex) and project to the substantia nigra pars compacta, where DA neurons are located (Bolam, Hanley, Booth, & Bevan, 2000; Gerfen, 1992; Haber, Kunishio, Mizobuchi, & Lynd-Balta, 1995). Due to the lack of specificity of the manipulation in the systemic approach, however, it is not clear whether the abnormal activity of the striosomes is the result of changes in cortical activity. It is also unclear whether intrinsic plastic changes within the striosomes are responsible for the observed behavior.

Electrophysiological studies have examined the effects of DA agonists on the firing properties of output neurons in the BG. They have found conflicting results depending on the method of administration. The systemic administration of apomorphine, which induces oral stereotypies in non-human primates, has revealed a net reduction in the firing rate in substantia nigra pars reticulata neurons (Nevet, Morris, Saban, Fainstein, & Bergman, 2004). With focal delivery of DA agonist into the striatum, however, the level of BG inhibitory output becomes highly variable, with a general net increase associated with stereotypic behavior (Waszczak et al., 2001; Waszczak, Martin, Finlay, Zahr, & Stellar, 2002). The latter observation following focal administration of DA agonists stands in contrast to an obvious decrease in activity following focal administration of GABA antagonists (see below) and those reported by Nevet et al. (2004) who used systemic apomorphine. Further work is necessary to clarify the mechanism by which different administration procedures cause opposing effects on BG output, which would then help identify the precise role of DA in fast network changes in tourettism.
3.1.2 Dopaminergic models for the premonitory urge

To date many of the available TS animal models emphasize face validity, focusing on the overt motor abnormalities, such as simple myoclonic-type tics and OCD-like behavior. As a result, these models fail to address one of the major symptoms of TS—the phenomenon of the premonitory urge (Swerdlow & Sutherland, 2005, 2006). The premonitory urge is described as an uncomfortable physical sensation that may feel like a crawling sensation or itch (Bliss, 1980; Kwak et al., 2003; Leckman, Walker, & Cohen, 1993). These sensory phenomena are relieved by performing a tic-related behavior in the locality of the sensation, for example, a cough in response to uncomfortable sensations in the throat. It has been hypothesized that sensorimotor gating may be abnormal in TS and can therefore be assessed by prepulse inhibition (PPI) of a startle response (e.g., blink reflex).

PPI is a behavioral phenomenon in which a weaker prestimulus (i.e., prepulse) inhibits the reaction to a subsequent strong stimulus (i.e., pulse) that can otherwise trigger a strong startle response. The stimuli are usually acoustic, but tactile or visual stimuli are also used. It has been shown that PPI is reduced in patients with TS (Castellanos et al., 1996; Swerdlow & Sutherland, 2005, 2006), suggesting that the ability of sensory stimuli to inhibit motor behavior is diminished. TS patients also show deficient intracortical inhibition and shortened cortical silent period as revealed by transcranial magnetic stimulation (Castellanos et al., 1996; Swerdlow et al., 2001; Ziemann, Paulus, & Rothenberger, 1997). Although not a direct model of TS, the PPI paradigm is a generalized testing protocol that can potentially be used across different experimental platforms and methodologies. It should also be recognized, however, that to date the relationship between PPI and premonitory urges has never been directly assessed.

DA agonists have effects on PPI performance. The focal administration of DA agonists in the limbic striatum produces deficits in sensorimotor gating, suggesting that this region and neurotransmitter may be involved in premonitory urges (Swerdlow et al., 2007; Wan, Geyer, & Swerdlow, 1995; Wan & Swerdlow, 1996). In rodents, systemic administration of DA agonists can induce variable effects depending on both the dosage and the strain of the animal. Specifically, the same dose can cause an increase or a decrease of PPI responses depending on the strain used (Rigdon, 1990; Swerdlow et al., 2007, 2003), while different dosages in the same animal strain can induce an increase or a decrease in the response depending on the stimulus condition. Obviously, these variable responses limit the conclusions that can be gained from this particular combination of DA models and PPI testing.
### 3.1.3 Serotonergic models for tic expression

Like DA, 5-HT is a monoamine neurotransmitter. 5-HT-containing neurons in the central nervous system are located in the raphe nuclei of the brainstem (Dahlstroem & Fuxe, 1964) and innervate virtually all of the brain structures (Dahlstroem et al., 1964). Pharmacological evidence suggests that a number of compounds that modulate 5-HT activity are effective for the treatment of tics, thus implicating the 5-HT system in tic pathogenesis. These compounds include serotonin reuptake inhibitors (Jimenez-Jimenez & Garcia-Ruiz, 2001; Silay & Jankovic, 2005) and atypical antipsychotics such as olanzapine, a neuroleptic drug that functions as an antagonist of 5-HT-2A/2C and D2 receptors (Budman, Gayer, Lesser, Shi, & Bruun, 2001); ondansetron, a 5-HT3 antagonist (Rizzo, Marino, Gulisano, & Robertson, 2008; Toren, Laor, Cohen, Wolmer, & Weizman, 1999); and tetrabenazine, an inhibitor of the 5-HT vesicular neurotransmitter transporter (Gros & Schuldiner, 2010; Jankovic & Orman, 1988).

A number of rodent animal models target the 5-HT system. In these models, movement disorders such as wet dog shakes and twitches as well as sensorimotor gating deficits can be induced via systemic injection of the precursor to serotonin 5-hydroxytryptophan (Bedard & Pycock, 1977; Corne, Pickering, & Warner, 1963) or systemic administration of 5-HT agonists (Kehne, Padi, McCloskey, Taylor, & Schmidt, 1996). Specifically for TS, 2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI), a 5-HT receptor agonist, induces head shakes and twitches (Colpaert & Janssen, 1983; Peroutka, Lebovitz, & Snyder, 1981) and has received particular attention as an animal model of TS (Handley & Dursun, 1992; Tizabi, Russell, Johnson, & Darmani, 2001). Although 5-HT models induce discrete tic-like movements, their frequency often occurs at very low rates, thus limiting the models applicability for electrophysiological studies. The tic-like movements induced by DOI have also been shown to be modulated by coadministration of cholinergic agonists (e.g., donepezil, an acetylcholine esterase inhibitor and nicotine, an agonist of nicotinic acetylcholine receptors) and DA antagonists (e.g., haloperidol). Correlated with the reduction of DOI-induced head twitches, nicotine increases 5-HT receptor expression in the striatum and cerebellum, but causes no change in midbrain receptor levels (Hayslett & Tizabi, 2005; Tizabi et al., 2001). Conversely, donepezil and haloperidol reduce DOI-induced head twitches and this reduction is correlated with decreased 5-HT levels in the frontal cortex (Hayslett & Tizabi, 2005). The DOI model suggests that the relative levels of neurotransmitters, and their effects on the up- and downregulation of receptors at anatomically distinct sites, are important factors in the pathogenesis of TS symptoms.
3.2. Intracerebral microinjection of pharmacological compounds

Focal, pharmacological targeting of areas in the cerebro-BG circuits is known to be effective for the induction of symptoms that, depending on the drug and functional division into which it is placed, bear a striking resemblance to the symptoms in TS. Although many active compounds can be used for this purpose, perhaps the most reliable ones are those that target local GABAergic transmission. Previous studies in rodents, felines, and nonhuman primates have established that focal disruption of GABAergic transmission in the cerebro-BG circuits can lead to the expression of hyperkinetic motor disorders. These abnormal movements, described in the literature as “repetitive myoclonic-type tics” or “choreic-like movements,” primarily involve single muscle pairs in the orofacial region (Fig. 7.1A), upper (Fig. 7.1B), and lower limbs (Crossman, Mitchell, Sambrook, & Jackson, 1988; Crossman, Sambrook, & Jackson, 1984; Gittis et al., 2011; Marsden, Meldrum, Pycock, & Tarsy, 1975; McCain, Bronfeld, Belelovsky, & Bar-Gad, 2009; Bronfeld, Belelovsky, & Bar–Gad, 2011; McCain, Iriki, & Isoda, 2013b; Muramatsu, Yoshida, & Nakamura, 1990; Tarsy, Pycock, Meldrum, & Marsden, 1978; Worbe et al., 2009). Focal microinjection of GABA antagonists, such as bicuculline, has several advantages when studying tic disorders. In particular, the effect is reversible with symptoms lasting about a few hours, the onset of symptoms is relatively rapid following drug delivery (typically 2–10 min), and the effect is repeatable by revisiting sites at a later date.

In the GABA antagonist model, simple motor tics can be induced through the microinjection of the drug to the sensorimotor circuits. Recent work has also shown that the same methodology, when targeting the limbic and associate territories, can induce complex motor behaviors. When the injections target the limbic region complex behaviors can have a number of discrete subtypes, but the most common responses are repetitive vocalization (Fig. 7.1C), chewing (Fig. 7.1D), intense grooming, and licking and biting of the fingers. When injections are placed into the associative territories the animals will display a behavioral abnormality whereby the animal does not show classical movement disorders, but instead will rapidly alternate between different actions that are part of the normal behavioral repertoire of the animal, often with hyperattraction to objects in the contralateral hemispace to the injection location. These complex behaviors and behavioral responses to injection have been suggested to be an analog of OCD (limbic) and ADHD (associative) symptomology (Baup et al., 2008; Francois et al., 2004; Grabli et al., 2004; Rotge et al., 2012; Worbe et al., 2009).
It is noteworthy that the same principle of methodology, that is, disruption of GABAergic processing, can induce phenomenologically different symptoms, with the only causal difference being the location of the injection within different functional territories of the cortico-BG circuits. This suggests that common pathophysiological mechanisms may underlie the multitude of abnormal behaviors in TS.

3.2.1 Electrophysiology of tics following localized GABA antagonist administration

Noninvasive neuroimaging techniques are a powerful tool to gain a global overview of brain regions exhibiting abnormal activation in disease conditions. In patients with TS, these techniques have successfully delineated the

![Figure 7.1](image-url)
extent of abnormalities, which encompasses not only the classical cerebro-BG structures (Peterson, 2001; Sowell et al., 2008; Worbe et al., 2010) but also the cerebellum (Bohlhalter et al., 2006; Lerner et al., 2007, 2012; Pourfar et al., 2011; Tobe et al., 2010). The importance of the cerebellum in the pathogenesis of the so-called BG disorders is increasingly recognized (Bostan & Strick, 2010; Wu & Hallett, 2013). Despite this progress, the fast neuronal changes in the global networks associated with tic disorders are poorly understood. Electrophysiological techniques should be able to address this issue, thereby complementing neuroimaging findings.

By identifying specific loci and patterns of neuronal activity that are causally related to TS symptoms, it should, in theory, be possible to target critical nodes within the tic-generating network for the suppression of pathological activity. It is now technically feasible to carry out simultaneous recording from individual neurons in the global cerebro–BG–cerebellar network during the expression of tics by using a nonhuman primate model produced by microinjection of GABA antagonists in the sensorimotor striatum. We now focus on this particular model to gain insights into the pathophysiology of tourettism and the mechanism of action of therapeutic deep brain stimulation.

### 3.2.2 Local field potential activity following microinjection of GABA antagonists

Following induction of motor tics via intrastriatal administration of GABA antagonists, the following changes have been reported in local field potentials (LFPs). Large “LFP spikes,” typically a few hundred milliseconds long, appear prior to and in conjunction with tic–related electromyographic (EMG) bursts. The LFP spikes appear at all the recorded nodes: the cerebral cortex, striatum, globus pallidus (GP), thalamus, and cerebellum (Fig. 7.2A) (Darbin & Wichmann, 2008; McCairn et al., 2009, 2013b; McKenzie & Viik, 1975; Muramatsu et al., 1990; Tarsy et al., 1978; McCairn et al., 2013b). Notably, it has been very recently reported that LFP spikes are present in the BG not only during overt tic expression, but also can occur during intertic intervals (Fig. 7.2A and 2Bi–iv) (McCairn et al., 2013b). It is possible that these intertic LFP spikes might be a neuronal correlate of the premonitory urge, which is a defining feature of TS.

In contrast, the emergence of LFP spikes in the cerebellum and the primary motor cortex (M1) is always confined to the period of overt tic movements. These observations suggest that the occurrence of tics at the behavioral level is more closely associated with the appearance of LFP spikes in the cerebellum and M1 (Fig. 7.2Bv–vi). This finding raises the possibility
Figure 7.2 Local field potential recordings during tic states. (A) Examples of raw data from EMG (top trace) and simultaneously recorded LFPs in putamen (dorsal and ventral regions), GPe, GPi, M1, and cerebellar cortex (CbllCx) after administration of bicuculline. EMG is recorded from the orofacial region. Gray shading shows periods of LFP spike activity in the basal ganglia in the intertic interval; note their absence in M1 and CbllCx. (B) Statistical comparison of averaged LFP spikes in the tic and intertic interval. (i) Average LFP spike amplitude in the dorsal putamen obtained during tic periods (red trace) and intertic interval (black trace). The inset plot shows statistical comparison of LFP spike shape; note the lack of significant difference between tic-associated LFPs and those in the intertic interval. (ii) Average LFP spike amplitude in the ventral putamen. Same conventions as in (Bi). (iii) Average LFP spike amplitude in the GPe. (iv) Average LFP spike amplitude in the GPi. (v) Average LFP spikes in M1. Note the presence of statistical difference in waveform structure between tic state and intertic interval (dashed line in the inset shows significance level $P=0.001$). (vi) Average LFP spikes in the CbllCx. Note the presence of statistical difference in waveform structure between tic state and intertic interval. Adapted from McCairn et al. (2013b).
that the cerebellum and M1 may function as a gate to trigger abnormal tic behavior and the presence of LFP spikes in these two structures can be the most reliable marker indicative of the physical occurrence of tics (McCairn et al., 2013b).

3.2.3 Spatial properties of injection effects within the striatum

Previous anatomical studies have suggested that there is a dorsal to ventral distribution of leg, arm, and then facial areas in the putamen (Crutcher & DeLong, 1984; Flaherty & Graybiel, 1991). A long-standing issue with respect to the microinjection protocol is why, when the injections are targeted to the dorsolateral regions (the area that corresponds to the leg), the effects are often manifested in the orofacial region. A potential explanation for this phenomenon may arise from the observation that, in the case of bicuculline, it induces excessive membrane depolarization at the immediate injection site (Feger, Vezole, Renwart, & Robledo, 1989), whereas increased single-cell activity can be found at the edge of the injection bolus (Worbe et al., 2009; Bronfeld et al., 2011). Supporting the observation of increased activity at regions distant to the injection site, recent studies have shown that there is a large difference in the amplitude of the LFP spikes between the dorsal (injection site) and ventral divisions of the putamen (McCairn et al., 2013b) (Fig. 7.2A). These differences in single-cell activity and LFP activity between locations inside and outside the injected site could account for the anatomical distribution of tic movement in the GABA antagonist model. In the model here, tics in the orofacial region are most reliably and strongly induced by the drug injected to the dorsal putamen, and their appearance is strongly associated with the appearance of LFP spikes that first emerge in the striatum and then propagate to other critical nodes in the cerebro-BG–cerebellar network.

3.2.4 Single-cell activity in the global neural network following microinjection of GABA antagonists

In parallel with LFP changes during tic movement, a number of effects can be seen at the single-cell level. These effects are characterized by phasic changes in firing rates at each recorded node, the latency of which systematically varies relative to EMG onset. In the striatum, at locations around the injection site, the predominant response is phasic activation of presumed medium spiny neurons. Their responses generally occur against a background of no activity in the intertic interval (Fig. 7.3A); however, it should be noted that like LFPs within the BG, it is possible to see intertic bursts of
single-unit activity which do not drive tic behavior (McCairn et al., 2013b),
these might be a correlate of the premonitory urge associated with TS. With
respect to single-unit onset latency, there is general agreement that striatal
activity occurs prior to cortical and EMG activity, with a latency difference
most typically being 0–200 ms (Bronfeld et al., 2011; McCairn et al.,
2013b). Another cell type in the striatum that shows tic-related activity is
the tonically active cholinergic interneurons (TANs) (Bronfeld et al.,
2011). The precise contribution of this cell type to the tic-generating
mechanism is as of yet unclear. Typically, TANs are thought to be involved
with reward processing and learning, and their firing is modulated via
DA transmission (Graybiel, Aosaki, Flaherty, & Kimura, 1994; Kimura,
Rajkowski, & Evarts, 1984). It has been shown that the timing of tic-related
TAN firing occurs on/or after the initiation of the tic events (Bronfeld et al.,
2011).

**Figure 7.3** Simultaneous recording of cerebro-basal ganglia–cerebellar activity during tics.
(A) Perievent raster and histogram of spiking activity in the striatum aligned to EMG onset.
(B) Perievent raster and histogram of a multiphasic GPe cell. (C) Perievent raster and histo-
gram for a tic-responsive GPI cell. Note the pronounced inhibition and latency relative to
EMG onset suggestive of a causal role in tic generation. (D) Perievent raster and histogram
for a tic-related M1 neuron. (E) Perievent raster and histogram for a tic-related cerebellar
cortex neuron. (F) Perievent raster and histogram for a tic-related dentate neuron.
This suggests that TANs could be influenced through a number of different pathways, including, but not limited to, tic/sensory-driven activation of DA projection neurons or via interaction with medium spiny neuron which shows strong phasic activations prior to tic activity.

The next critical nodes in the network downstream from the striatum are the two segments of the GP. In response to tic events, neurons in the GP, like those in the striatum, also show phasic changes in activity. The typical response in the external segment of GP (GPe) is excitation, with some multiphasic (Fig. 7.3B) or inhibitory responses also present, while the internal segment (GPi) predominantly expresses inhibition (Fig. 7.3C), with some multiphasic and excitatory responses also detectable (McCairn et al., 2009; McCairn, Iriki, & Isoda, 2012, 2013a; McCairn et al., 2013b; Muramatsu et al., 1990; Bronfeld et al., 2011). These responses, in terms of response sign, match predictions from theoretical models of the BG. Specifically, during overt movement, the GPe is predicted to increase its activity while the GPi is supposed to decrease its activity, leading to disinhibition of the thalamocortical projection (Albin, Young, & Penney, 1989, 1995; DeLong, 1990). If “action selection” models of BG function are correct, especially with respect to tic generation, then timing of pallidal activity should occur prior to tic initiation in a focal manner. Studies utilizing EMG activity as a marker for tic initiation suggested that GPi activity is early enough to be causal to tic generation (McCairn et al., 2009). A later study, however, which compared GPi inhibition to the onset of tic–related activity in M1 reports that GPi inhibition is concurrent with M1 activity and is therefore too late to be involved in tic initiation (Bronfeld et al., 2011). This particular observation leads to a breakdown of the causal pathway from the site of the injection (i.e., striatum) to the final output of the circuit (i.e., M1). In order to address this curious result, Bronfeld and colleagues propose a model in which loss of anatomical specificity in the BG and its effect on intra- and internuclear information processing is the key mechanism for tic generation (Bronfeld & Bar–Gad, 2011; Bronfeld et al., 2011).

More recent studies, however, have managed to identify early responding cells in the GP relative to activity in M1, thus preserving a causal chain from the BG to the output motor centers (McCairn et al., 2013b). This recent work would appear to partially support the premise of aberrant BG–mediated action selection (Albin & Mink, 2006; Mink, 2001) as the mechanism underlying tics. In contrast to the focal action selection hypothesis, however, the results from the microinjection experiments show that the abnormalities in the GP are not focal in nature, because as many as 70–80%
of neurons in each GP segment covering large regions of the nucleus display tic-related activity (McCairn et al., 2009, 2013b; Bronfeld et al., 2011; Muramatsu et al., 1990). It is therefore difficult to reconcile the focal nature of the tic, which generally confines to one muscle group, with the large area of activation within the BG. A tempting hypothesis is that early responding neurons described earlier (McCairn et al., 2013b), which are small in number and focally confined, may correspond to the core area for tic expression and they have a “winner-takes-all” effect on motor output centers. This would then lead to a localization of the tic, while other surrounding regions corresponding to other anatomical localities can display tic-related firing patterns without affecting behavior.

Following the pathway of tic propagation, downstream to the BG output is the thalamus and motor cortices. At the level of the thalamus it has been reported that pallidal receiving areas show increased excitation (Muramatsu et al., 1990), which is consistent with disinhibition following reduced activity in the GPi. This increased thalamic activity is also associated with phasic increases in M1 that typically occur 0–90 ms prior to tic initiation (Fig. 7.3D) (McCairn et al., 2009, 2013b; Bronfeld et al., 2011; Muramatsu et al., 1990).

3.2.5 Fast latency neuronal responses outside the classical cortico-BG networks

Emerging evidence from neuroimaging studies has suggested that the cerebellum may also play a role in the pathogenesis of TS (Bohlhalter et al., 2006; Lerner et al., 2007, 2012; Pourfar et al., 2011; Tobe et al., 2010). In support of this view, anatomical studies have identified subcortical pathways linking the BG and cerebellum disynaptically (Bostan, Dum, & Strick, 2010; Bostan & Strick, 2010; Hoshi, Tremblay, Feger, Carras, & Strick, 2005) (Fig. 7.4A). Indeed, a recent electrophysiological study has shown that a sizable number of cerebellar neurons, both in the cortical and deep nuclear divisions, show phasic changes in activity in the bicuculline-induced tic model (McCairn et al., 2013b). A critical observation from the study was that abnormal discharges of cerebellar cortex neurons and excitatory-type dentate neurons mostly preceded behavioral tic onset, indicating a central origin of their activation (Fig. 7.3E and F). Of particular interest was the observation that the latency of pathological activity in the cerebellum and M1 substantially overlapped and are statistically indistinguishable (McCairn et al., 2013b). This suggests that aberrant signals may be traveling along divergent pathways to these structures from the BG. These findings, in conjunction with LFP spikes in the
Figure 7.4 Cerebro-basal ganglia–cerebellar basis of tics. (A) Simplified circuit diagram showing the synaptic interactions between the cerebral cortex, basal ganglia, and cerebellum. Note the presence of the subcortical projections between the basal ganglia and cerebellum. STR, striatum; GPe, globus pallidus externus; GPi, globus pallidus internus; STN, subthalamic nucleus; PN, pontine nucleus; CbCx, cerebellar cortex; Den, dentate; HTL, thalamus. (B) Schematic drawing of the relative population response of single units in the cerebro-basal ganglia–cerebellar network.
cerebellum mentioned earlier, indicate that tic-generating networks extend beyond the classical cerebro-BG circuits, leading to the hypothesis that tic expression can be considered a form of “global network dysrhythmia” including cerebellar circuits. The relative response type of each critical node in the cerebro-BG–cerebellar network is shown in Fig 7.4B.

Another important insight from the bicuculline model is the involvement of GABAergic transmission in the pathogenesis of TS disorders. The emphasis on GABAergic networks has recently been corroborated by imaging studies in TS patients (Lerner et al., 2012). Postmortem examination has also identified a reduced expression of fast spiking inhibitory GABAergic neurons in TS patients (Kalanithi et al., 2005; Kataoka et al., 2010). These findings raise the possibility that the underlying cause of TS may not be a disorder of monoamines such as DA and 5-HT, but instead reside in abnormal GABAergic processing. This hypothesis is also supported by the observation that GABA agonists such as clonazepam and baclofen can ameliorate symptoms of TS (Goetz, 1992; Singer, Wendlandt, Krieger, & Giuliano, 2001). Future work should address whether, and how if any, the manipulation of GABAergic processing affects transmission in the DA system, and vice versa.

3.2.6 Neurosurgical intervention in GABA antagonist models

An important component of any animal model is that it provides a platform to test the properties of therapeutic intervention. An emerging experimental treatment for TS is a neurosurgical intervention utilizing high-frequency deep brain stimulation (HF-DBS). This therapeutic intervention typically targets critical nodes in the cortico-BG–thalamic circuits (Houeto et al., 2005; Krack, Hariz, Baunez, Guridi, & Obeso, 2010; Martinez-Fernandez et al., 2011; Mink, 2009; Saleh, Gonzalez, Cif, & Coubes, 2012; Welter, Grabli, & Vidailhet, 2010). There is still, however, a vigorous debate with respect to the optimal site for placement of DBS electrodes. Current claims from various centers suggest that both segments of the GP, the GPe and GPi (Dehning, Mehrkens, Muller, & Botzel, 2008; Dueck et al., 2009; Houeto et al., 2005; Martinez-Fernandez et al., 2011; Piedimonte et al., 2012; Shahed, Poysky, Kenney, Simpson, & Jankovic, 2007; van der Linden et al., 2002; Welter et al., 2008), the subthalamic nucleus (STN) (Martinez-Torres, Hariz, Zrinzo, Foltynie, & Limousin, 2009), and the thalamus (Vandewalle, van der Linden, Groenewegen, & Caemaert, 1999) are all potential sites for therapeutic stimulation, for review see Ackermans and colleagues (Ackermans, Kuhn, Neuner, Temel, & Visser-Vandewalle, 2012).
The GABA antagonist model described earlier provides an opportunity to quantitatively assess the efficacy of HF-DBS in alleviating tic symptoms and to study the neurophysiological correlates of any therapeutic response. Recently, use of the localized GABA antagonist model has resulted in the publication of three reports on the effect of HF-DBS in the nonhuman primate platform. In one study, where bicuculline was targeted to the limbic pallidum, the incidence of stereotypic behavior was reduced following the onset of HF-DBS in the STN (Baup et al., 2008). Two more recent studies (McCairn et al., 2012, 2013a) have shown that HF-DBS in the GPi reduces the amplitude of simple motor tics that are induced by bicuculline microinjection into the sensorimotor striatum (Fig. 7.5A). Targeting HF-DBS to the output nuclei of the BG was capable of modulating the firing properties of single cells (Fig. 7.5B) in both segments of the pallidum. This modulation led to a suppression of the phasic tic-related alterations in firing rate which drive tics in both the GPe (Fig. 7.5C) and the GPi (Fig. 7.5D). Analysis of the short-term interactions between deliveries of stimulus pulses found that there was a temporal locking of the cell’s spiking activity, which induced complex patterns of excitation and inhibition (Fig. 7.5E and F). Similar entrainment of pallidal output has also been observed in parkinsonian models that have used GPi or STN stimulation (Chiken & Nambu, 2013; Erez, Czitron, McCairn, Belelovsky, & Bar-Gad, 2009; Hanson & Jaeger, 2002; Hashimoto, Elder, Okun, Patrick, & Vitek, 2003; McCairn & Turner, 2009; Meissner et al., 2005; Moran, Stein, Tischler, Belelovsky, & Bar-Gad, 2011). This suggests that in BG mediated hypo- and hyperkinetic disorders HF-DBS has a common therapeutic mechanism.

4. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

For many years, the dopaminergic theory has dominated the pathophysiology of TS and deficient cortico-BG networks have been implicated in TS symptomatology. Many of the current, pharmacological animal models have been developed on the basis of these premises. However, recent progress in neurochemistry, neurophysiology, and neuroimaging now prompts us to have a different picture of the biological and network mechanism of TS. It is becoming clear that aberrant GABAergic transmission is crucially involved, and the cerebellum outside the classical cortico-BG circuits also participates in the pathogenesis of TS. In light of these conceptual advances, the focal GABA antagonist model appears to offer
Figure 7.5 Testing deep brain stimulation in the focal GABA antagonist model. (Ai) Examples of raw EMG (orofacial region) after administration of bicuculline and during HF-DBS; the large voltage transients are tic events. (Aii) The reduction of peak voltage amplitude in tic-related EMG. The histogram shows the EMG signals aligned to tic onset.

(Continued)
a promising avenue for the understanding of tic disorders. It meets face validity (simple motor tics combined with OCD/ADHD-like behavior depending on the site and extent of injection), construct validity (impaired local GABA transmission leading to cerebro-BG–cerebellar network dysrhythmia), and predictive validity (responsiveness to deep brain stimulation). For further refinement of the existing tic model, it would be of great importance to combine different domains of model platforms (e.g., genetic, pharmacological, and environmental) given the complex etiology and phenotype of TS.

REFERENCES


