

Control of trigeminal motoneuron behavior and masseter muscle tone during REM sleep, REM sleep behavior disorder and cataplexy/narcolepsy

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ABSTRACT

REM sleep triggers a potent suppression of postural muscle tone – i.e., REM atonia. However, motor control during REM sleep is paradoxical because overall brain activity is maximal, but motor output is minimal. The skeletal motor system remains quiescent during REM sleep because somatic motoneurons are powerfully inactivated. Determining the mechanisms triggering loss of motoneuron function during REM sleep is important because breakdown in REM sleep motor control underlies sleep disorders such as REM sleep behavior disorder (RBD) and cataplexy/narcolepsy. For example, RBD is characterized by dramatic REM motor activation resulting in dream enactment and subsequent patient injury. In contrast, cataplexy – a pathognomonic symptom of narcolepsy – is caused by the involuntary onset of REM-like atonia during wakefulness. This review highlights recent work from my laboratory that examines how trigeminal motoneuron activity is lost during normal REM sleep and it also identifies potential biochemical mechanisms underlying abnormal motor control in both RBD and cataplexy. First, I show that neither GABA_A/glycine mediated inhibition of motoneurons is required for generating REM atonia. Instead, our preliminary data suggest that both metabotropic GABA_B and ionotropic GABA_A/glycine inhibition of trigeminal motoneurons is required for activating REM atonia. Next, I show that impaired GABA and glycine neurotransmission triggers the cardinal features of RBD in a transgenic mouse model. Last, I discuss our recent, unpublished data that suggests that loss of an excitatory noradrenergic drive onto motoneurons is, at least in part, responsible for the loss of postural muscle tone during cataplexy in narcoleptic mice. Together, this research indicates that multiple transmitters systems are responsible for regulating postural muscle tone during REM sleep, RBD and cataplexy.

Key words

Sleep • Motor control • REM sleep • Motoneuron • Trigeminal • Narcolepsy • Cataplexy • REM sleep behavior disorder

Introduction

Sleep markedly affects fundamental mechanisms of motor control. Sleep not only suppresses postural muscle tone (Brooks and Peever, 2008b; Burgess et al., 2008), but it also attenuates, and in some cases even abolishes motor reflexes (e.g., H-reflex) (Wills

and Chase, 1979). Mechanisms of motor control are also differentially affected by prevailing behavioral state. For example, muscle tone and motor reflexes are reduced during NREM sleep, but virtually eliminated during REM sleep (i.e., REM atonia) (Burgess et al., 2008). However, motor control during REM sleep is unique because it is characterized by flur-

ries of periodic muscle twitches that punctuate REM sleep atonia (Brooks and Peever, 2008b; Burgess et al., 2008). This review highlights recent work from my laboratory that examines mechanisms controlling trigeminal motoneuron function and muscle tone during REM sleep.

Motor control during REM sleep is paradoxical because muscle tone and movement are largely absent, despite the fact that overall brain activity, even in locomotor-generating regions, is maximal. The skeletal motor system is able to disconnect itself from the REM-active brain by reducing somatic motoneuron activity and hence motor tone. Determining mechanisms mediating motoneuron behavior during REM sleep is of direct clinical relevance because abnormal motor control during REM sleep underlies major sleep disorders such as REM sleep behavior disorder (RBD) and narcolepsy/cataplexy. In this review, I also describe recent advances in our understanding of motor control and motoneuron function in animal models of both RBD and narcolepsy/cataplexy.

A long-standing hypothesis has been that loss of postural muscle tone during REM sleep is *only* caused by inhibition of motoneurons (Chase, 2008; Soja, 2008). However, multiple lines of electrophysiological, biochemical and immunohistochemical evidence from several different laboratories now indicate that REM motor activity is suppressed by both direct inhibition and passive disfacilitation of motoneuron activity (Chase et al., 1989; Kubin et al., 1994; Lai et al., 2001; Fenik et al., 2005; Sood et al., 2005; Brooks and Peever, 2008b; Burgess et al., 2008; Schwarz and Peever, 2010). Even though both inhibition and reduced excitation mediate motoneuron activity in REM sleep, this review primarily highlights our recent work examining the roles that GABA and glycine neurotransmission play in triggering REM motor atonia.

Mechanisms controlling muscle tone during REM sleep

Until recently, a dominating theme in sleep physiology has been that REM atonia is only triggered by glycinergic inhibition of motoneurons (Chase, 2008; Soja, 2008). This hypothesis was initially proposed by Dr. Chase and stemmed from his intracellular

studies of trigeminal and lumbar motoneurons during REM sleep in head-restrained cats (Nakamura et al., 1978; Soja et al., 1991). Although the intracellular approach is important for determining aspects of synaptic physiology, it does not actually allow one to determine if glycine inhibition of motoneurons actually causes *physiological* changes in muscle tone. Indeed, both Drs. Horner and Kubin showed that preventing glycine-mediated inhibition at the entire hypoglossal motor nucleus does not reverse the normal loss of genioglossus muscle tone during either natural or drug-induced REM sleep (Kubin et al., 1993; Morrison et al., 2003). Their results therefore called into question the role that glycinergic inhibition plays in generating REM sleep atonia.

Because of the discrepancy between initial intracellular recordings and results from Drs. Horner's and Kubin's laboratory, we re-examined the results of the original intracellular data (Soja et al., 1991). We noted several observations that might explain why blocking glycine receptors on motoneurons may not prevent REM atonia. Although intracellular recording experiments demonstrate that motoneurons are inhibited by glycine, there is evidence that non-glycinergic mechanisms are also involved. For example, strychnine (glycine receptor antagonist) blocked inhibitory post-synaptic potentials (IPSPs) in only 32% (6 of 19 cells) of recorded lumbar motoneurons during REM sleep; IPSPs remained, albeit with reduced amplitudes and frequencies, in 68% of recorded cells (Soja et al., 1991). It is unlikely that residual IPSPs were the result of inadequate glycine receptor antagonism because the dose of strychnine that Dr. Chase used (i.e., 15 mM) was sufficient for blocking glycine receptors on motoneurons *in-vitro* (Jonas et al., 1998; O'Brien and Berger, 1999). Therefore, although intracellular studies illustrate that glycine inhibits motoneurons during REM sleep, it would appear that it is not the exclusive factor responsible for either generating IPSPs or for hyperpolarizing motoneurons during REM sleep. Other biochemical mechanisms appear to also play a role in generating inhibition/hyperpolarization – mechanisms that require identification.

My laboratory therefore set out to identify the potential mechanisms that trigger motoneuron inhibition and hence REM atonia. We chose to examine the trigeminal-masseteric motor system because masseter muscle tone is potentially suppressed during REM

sleep (Brooks and Peever, 2010; Schwarz and Peever, 2010) and abnormal masseter function contributes to the pathogenesis of several sleep disorders including RBD, obstructive sleep apnea, cataplexy/narcolepsy and bruxism (Guilleminault et al., 1974; Soja et al., 1987; Horner, 1996; Schenck and Mahowald, 2002; Kato et al., 2003). In addition, the glycinergic (and GABAergic) control of individual trigeminal motoneurons has been well documented during REM sleep using the intracellular recording technique (Soja et al., 1987; Chase et al., 1989). In the experiments I describe below, we implemented a variety of approaches, including reverse-microdialysis, electrophysiology, receptor pharmacology and genetics to determine how manipulation of neurotransmission at the trigeminal motor pool affects motoneuron function and hence motor outflow to masseter muscles during REM sleep in both rats and mice.

Pharmacological blockade of glycine and GABA_A receptors on trigeminal motoneurons does not prevent REM atonia

If a functional glycinergic drive underlies loss of muscle tone in REM sleep (Chase et al., 1989; Soja et al., 1991), then antagonism of glycine receptors on trigeminal motoneurons should reverse masseter REM sleep atonia. However, we show that glycine receptor antagonist (0.1 mM strychnine) at the trigeminal motor pool never had any effect on levels of masseter tone during tonic REM sleep even though it increased basal masseter tone during both waking and NREM sleep (Brooks and Peever, 2008b). Although REM atonia was left untouched by receptor inactivation, it nonetheless triggered potent increases the amplitude of muscle twitches during phasic REM sleep (Brooks and Peever, 2008b). Together these results indicate that 1) a tonic glycine drive inhibits motoneurons during both waking and NREM sleep, 2) a phasic glycinergic drive acts to suppress muscle twitches during REM sleep, and 3) that glycine inhibition of motoneurons is not sufficient to trigger REM atonia. As previously indicated, similar results have been earlier obtained at the hypoglossal motor pool (Kubin et al., 1993; Morrison et al., 2003) and together these results call in question the exclusive role of glycine in causing motoneuron inhibition and hence REM atonia.

Because motoneurons are concurrently inhibited by GABA and glycine during REM sleep (Soja et al.,

1987) and because both transmitters are coreleased onto motoneurons (O'Brien and Berger, 1999) during pharmacologically induced REM-like sleep (Kodama et al., 2003), we hypothesized that both GABA_A and glycine transmission may be required for producing atonia. Therefore, we simultaneously antagonized glycine and GABA_A receptors in the trigeminal motor pool during REM sleep. We found that strychnine and bicuculline (GABA_A receptor antagonist) perfusion increased masseter tone during both waking and NREM sleep; however, it was unable to reverse REM atonia (Fig. 1) (Brooks and Peever, 2008b). Such results suggest that neither GABA_A nor glycine-mediated inhibition of motoneurons are sufficient for triggering atonia. It should also be noted that neither glycine nor GABA_A mediation inhibition of hypoglossal motoneurons is sufficient for suppressing genioglossus muscle activity during natural REM sleep (Morrison et al., 2003).

Because the preceding experiments show that REM atonia could not be reversed by blockade of glycine and GABA_A receptors (Fig. 1), we hypothesized that atonia may be mediated by a disfacilitation of excitatory inputs onto motoneurons (Fenik et al., 2005; Chan et al., 2006). Therefore, we antagonized both glycine and GABA_A receptors while simultaneously activating trigeminal motoneurons with a potent dose of AMPA (0.1 mM), a glutamate receptor agonist. Intriguingly, we found that this intervention had powerful stimulatory effects on masseter tone during both waking and NREM sleep; however, this excitatory effect was immediately abolished at the transition from NREM sleep into REM sleep, with REM atonia persisting despite simultaneous antagonism of both glycine and GABA_A receptors and activation of AMPA receptors (Brooks and Peever, 2008b). Together, these experiments indicate that a powerful, yet unidentified, inhibitory mechanism must override trigeminal motoneuron excitation during REM sleep.

Before identifying the potential inhibitory mechanism(s) responsible for this source of motoneuron inhibition, I highlight and summarize an additional series of experiments that aimed to further determine the extent to which GABA_A and glycine receptor-mediated inhibition play in initiating REM atonia. These experiments took advantage of a transgenic mouse model that has reduced glycine and GABA_A receptor function.

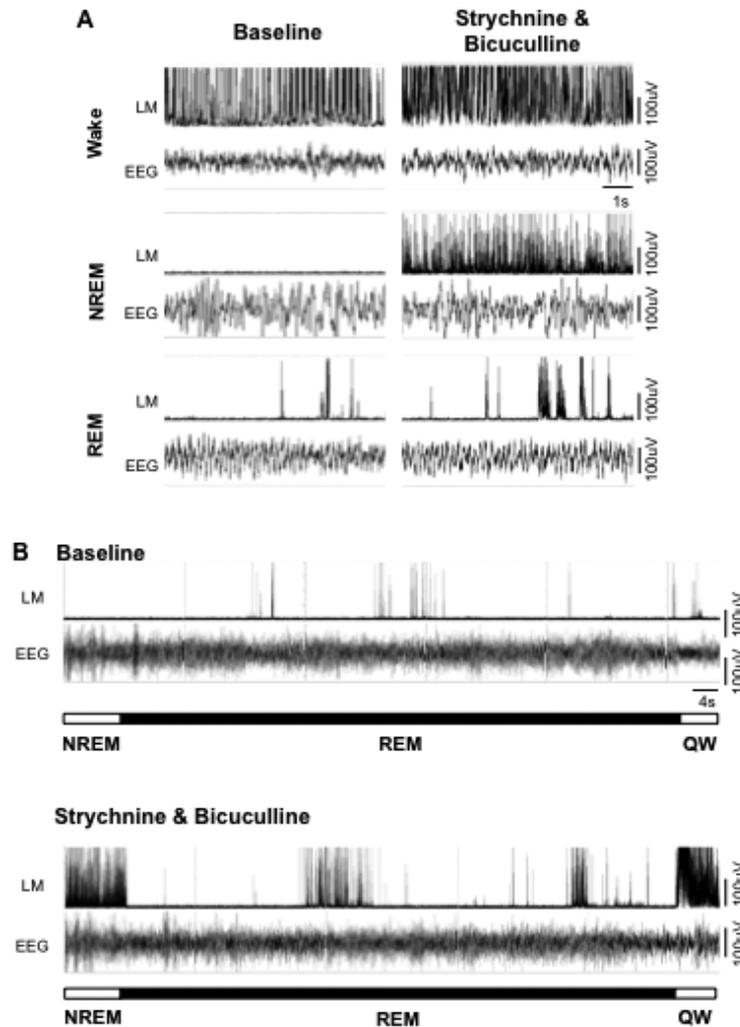


Fig. 1. - Blockade of glycine and GABA_A receptors at the trigeminal motor pool does not prevent masseter muscle atonia during REM sleep. A. Typical EMG and EEG traces illustrating that strychnine/bicuculline perfusion into the left trigeminal motor pool increases left masseter muscle (LM) tone during waking and NREM sleep; this intervention only provokes muscle twitch activity during REM sleep, it does not reverse masseter REM atonia. B. Example EMG/EEG traces showing how left masseter muscle activity changes during the transition into and out of REM sleep during baseline and strychnine/bicuculline perfusion. Traces illustrate that strychnine/bicuculline perfusion at the left trigeminal motor pool increases LM activity during the NREM and waking periods preceding and following REM onset, but that masseter atonia is unaffected by this intervention. Modified from Brooks and Peever (2008b).

Deficient GABA_A and glycine receptor function does not prevent REM sleep atonia

Here, I highlight results from transgenic mice that have impaired glycine and GABA_A receptor function (Becker et al., 2002; O'Shea et al., 2004; Brooks and Peever, 2011). We studied these mice for two separate, but overlapping reasons. First, we wanted to further define the role for GABA and glycine transmission in producing REM atonia, and second, we aimed to determine if impaired inhibi-

tion could trigger a motor phenotype that mimics human RBD.

Transgenic mice were generated by expressing a mutant glycine receptor α_1 subunit in the mouse genome; mutant receptors were expressed throughout the central nervous system (Becker et al., 2002; O'Shea et al., 2004). *In vitro* cell recordings show that transgenic spinal neurons not only experience a 70% reduction in glycine receptor-mediated inhibition, but they also exhibit a 91% reduction in GABA_A receptor-mediated inhibition (Becker et al.,

2002). The exact cause of reduced GABA_A-mediated inhibition is unknown, but nonetheless transgenic mice experience a potent reduction in both glycine and GABA_A receptor-mediated inhibition (Becker et al., 2002). These mice therefore serve as a tool for assessing how deficits in inhibitory transmission affect REM atonia. Despite almost complete loss of normal GABA_A and glycine mediated inhibition, electrophysiological (i.e., EEG and EMG) and behavioral data show that REM atonia is preserved in masseter, neck and hindlimb muscles in transgenic mice (Brooks and Peever, 2011). However, we found that these transgenic mice have a marked exaggera-

tion of muscle twitches during REM sleep episode despite maintenance of REM atonia (Fig. 2) (Brooks and Peever, 2011). These results further indicate that GABA_A and glycine neurotransmission play a negligible role mediating the loss of postural muscle tone in REM sleep.

Deficient GABA_A and glycine receptor function triggers an RBD phenotype in transgenic mice

Here, we aimed to determine if impaired inhibition could trigger a motor phenotype that mimics human RBD. Patients with RBD are neurologically and

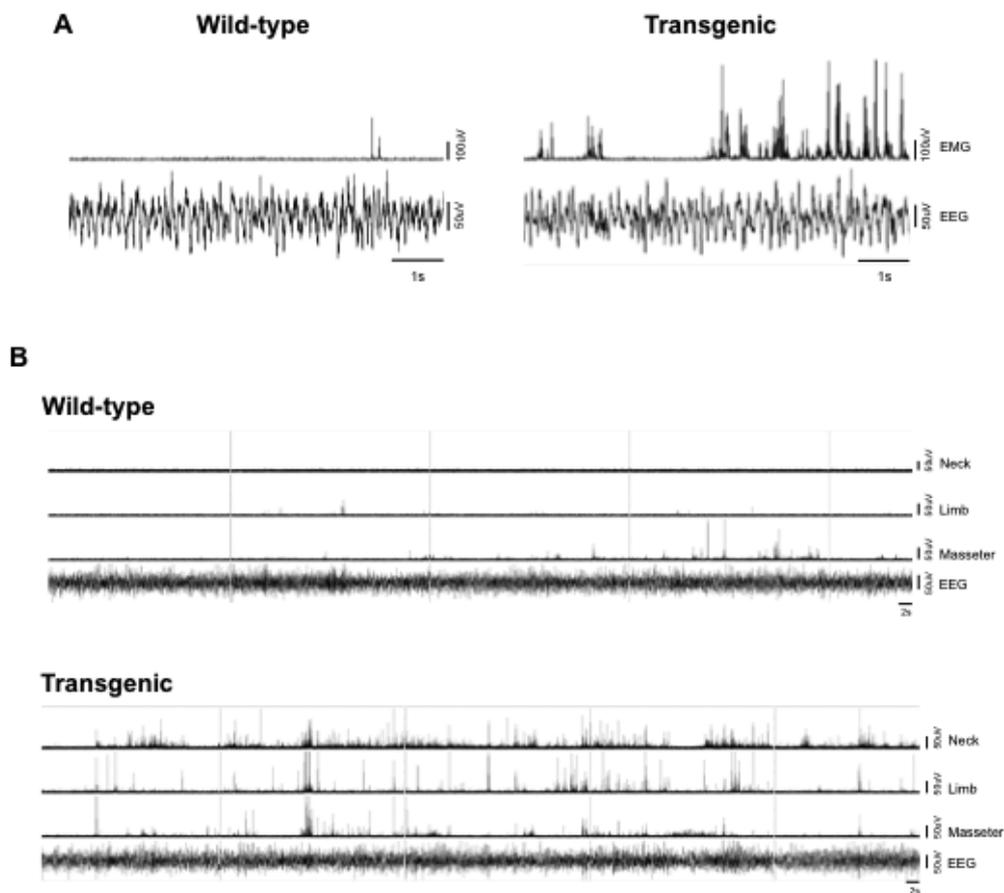


Fig. 2. - Transgenic mice with deficient GABA and glycine receptor function still exhibit REM atonia, but have heightened REM twitch activity. A. High resolution snap-shot of masseter EMG and EEG traces from a wild-type (top trace) and transgenic mouse (bottom trace) during REM sleep. In contrast to wild-type mice, transgenic mice experience a marked increase in muscle twitches during REM sleep even though REM atonia is preserved. B. EMG and EEG traces recorded from an entire REM episode in a wild-type (top traces) and transgenic mouse (bottom traces). EMG traces were recorded from neck, hindlimb and masseter muscles and demonstrate that transgenic mice suffer from pronounced activation of motor activity in REM sleep. This activity results from exaggeration of phasic motor activity; periods of REM atonia remain intact. Such motor activation resulted in REM motor behaviors such as running and chewing. Transgenic mice, therefore, exhibit a motor phenotype that mimics human RBD.

motorically normal during waking, but they have severe disruption of motor function during REM sleep (Mahowald and Schenck, 2005). RBD is typified by REM motor activation and violent dream enactment; however, muscle twitches and limb jerks during NREM sleep, sleep disruption and slowing of the EEG are also defining disease symptoms (Sforza et al., 1997; Olson et al., 2000; Schenck and Mahowald, 2002).

RBD is clinically important for two reasons. First, REM motor activation and repetitive NREM muscle jerks disrupts sleep continuity, and violent dream enactment often results in bodily injuries – lacerations, fractures and hospital visits are common in RBD. Second, a majority of RBD patients develop Parkinson's, multiple system atrophy or dementia with Lewy bodies within 12 years of initial diagnosis (Schenck et al., 1996; Iranzo et al., 2006). RBD is therefore a harbinger of some neurodegenerative diseases.

Although the exact cause of RBD is unknown, abnormal glycine and GABA neurotransmission is implicated in the disorder. First, strokes and lesions that affect brainstem regions containing glycine and GABA neurons triggers motor activation during REM sleep (Schenkel and Siegel, 1989; Lu et al., 2006; Boeve et al., 2007). Second, patients with impaired glycine and GABA transmission often experience heightened motor activity during sleep (de Groen and Kamphuisen, 1978; Martinelli et al., 1996). Third, drugs that strengthen inhibitory function (e.g. clonazepam and melatonin) are the most common and effective treatment for RBD motor symptoms (Olson et al., 2000; Schenck and Mahowald, 2002).

Intriguingly, we found that transgenic mice with deficient glycine and GABA transmission not only have REM motor activation, they also have the full complement of RBD symptoms that define the human disorder. Specifically, we found that all transgenic mice exhibit complex motor behaviors during REM sleep—running, jerking and chewing are common behaviors (Brooks and Peever, 2011). This is in sharp contrast to their wild-type littermates, which remained motorically quiescent during REM episodes. Exaggerated muscle twitches that typify normal REM sleep were the primary trigger of heightened muscle activity and hence RBD behaviors in these mice (Fig. 2) (Brooks and

Peever, 2011). Even though transgenic mice had increased phasic motor activity, they had normal levels of basal muscle tone, i.e., REM atonia (Fig. 2). Excessive muscle twitch activity during REM sleep is reminiscent of human RBD and, therefore, recapitulates the primary human disease symptom. Transgenic mice also displayed other disease features that typify human RBD. For example, they experience brief, repetitive EMG twitches and limb jerks during NREM sleep. The jerks/twitches that dominated NREM sleep in these mice were episodic in nature, with 70% of them occurring approximately every 20 s (Brooks and Peever, 2011). This frequency and periodicity is similar to that in human RBD patients (Schenck and Mahowald, 2002).

Transgenic mice also suffer from pronounced sleep fragmentation. They awoke 135% and 34% more from NREM and REM sleep episodes than wild-type mice. Increased arousals had an impact on sleep-wake amounts and architecture, increasing wakefulness and severely suppressing NREM sleep amounts, even though total amounts of REM sleep were unaffected. Fragmentation of NREM sleep was not caused by NREM motor activation because only 10% of arousals were preceded (within 5 s) by muscle twitches, i.e., 90% arousals were triggered by non-motor events.

Transgenic mice also exhibited slowing in certain EEG frequencies, which is also a common feature in human RBD (Gagnon et al., 2006). Although gross EEG examination revealed no difference between wild-type and transgenic mice in waking or sleep, spectral analysis revealed greater EEG power in the theta range (i.e., 5-7 Hz) in transgenic mice, i.e., an indication of EEG slowing. This cortical slowing is prominent during both waking and NREM sleep but not REM sleep. These findings mimic the EEG phenotype observed in human RBD patients (Gagnon et al., 2006).

Importantly, the RBD motor phenotype is rescued by drugs (e.g., clonazepam and melatonin) that are routinely used to treat human disease symptoms (Schenck and Mahowald, 2002). We found that clonazepam reduced overall EMG tone by 26% during REM sleep; it also reduced NREM muscle twitches by 51% (Brooks and Peever, 2008a, 2011). We also found that melatonin treatment reduced muscle twitches in REM sleep by 43% and improved sleep by alleviating sleep fragmentation (Brooks and Peever, 2008a, 2011).

Together these data show that impaired inhibitory transmission triggers the hallmark features of RBD in transgenic mice. These findings not only identify a potential genetic mechanism for RBD, they also indicate that transgenic mice could serve as a resource for determining RBD pathogenesis. Although previous animal studies identify brainstem circuits underlying abnormal REM motor control (Jouvet and Delorme, 1965; Hendricks et al., 1982; Friedman and Jones, 1984; Schenkel and Siegel, 1989; Shouse and Siegel, 1992; Holmes and Jones, 1994; Sanford et al., 2001; Lu et al., 2006; Vetrivelan et al., 2009), this new mouse model represents an important advance because it recapitulates *all* primary RBD features stemming from a defined genetic mutation. We propose that transgenic mice are a useful model for determining how glycine and GABA transmission contribute to RBD development. Our results also emphasize the need to determine if impaired glycine and GABA transmission also contribute to motor and sleep symptoms in human RBD (Brooks and Peever, 2011).

Are metabotropic GABA_B receptors important for triggering REM atonia?

Finally, I discuss our unpublished data supporting the potential role that GABA_B receptors might play in eliciting REM atonia (Brooks, 2009). Although metabotropic GABA_B receptors function to mediate post-synaptic inhibition (Bormann, 1988), their role in mediating REM atonia is unknown. However, multiple lines of evidence indicate that GABA_B receptor-mediated inhibition could regulate motoneuron behavior and muscle tone. First, biochemical studies show that GABA (and glycine) is released onto motoneurons in both spinal and cranial motor pools during REM-like sleep (Kodama et al., 2003). Second, GABAergic neurons project to and synapse on somatic motoneurons (Chase et al., 1984; Li et al., 1996), which themselves express GABA_B receptors (Towers et al., 2000; Charles et al., 2003). Third, GABA_B receptor activation elicits robust hyperpolarization of motoneurons (and respiratory-related neurons) and inhibits their activity/output (Lalley, 1986; Haji and Takeda, 1993; Okabe et al., 1994; O'Brien et al., 2004). We therefore hypothesize that REM atonia could be triggered, at least in part, by GABA_B receptor-mediated inhibition. Our current preliminary data suggest that REM

atonia is caused by activation of both metabotropic GABA_B and ionotropic GABA_A/glycine receptors on trigeminal motoneurons. Using approaches similar to those described in *section 3a*, we found that pharmacological antagonism of both GABA_B and GABA_A/glycine receptors at the trigeminal motor pool triggered potent increases in basal masseter tone during REM sleep (Brooks, 2009). This is in clear contrast, to blockade of either GABA_A/glycine receptors (Kubin et al., 1993; Morrison et al., 2003; Brooks and Peever, 2008b) or GABA_B receptors only (our unpublished data), which had no effect on REM motor tone. These, albeit unpublished findings, suggest that together GABA_B and GABA_A/glycine receptor-mediated inhibition of motoneurons is important for triggering REM atonia. Accordingly, we suggest that multiple inhibitory mechanisms are responsible for eliciting REM sleep atonia.

Mechanisms mediating motor atonia during cataplexy

Cataplexy is characterized by a sudden, involuntary loss of skeletal muscle tone during wakefulness and it is a pathognomonic symptom of the sleep disorder narcolepsy (Scammell et al., 2009). It is generally triggered by strong positive emotions such as laughter or intense excitement (Krahn et al., 2005). The motor atonia of cataplexy can last from several seconds to several minutes, and in some cases can be followed by direct entrance into REM sleep (Overeem et al., 1999). Because motor atonia is a defining feature of both cataplexy and REM sleep similar neuro-circuits and biochemical mechanisms may mediate both motor phenomena. Here, I briefly discuss some of our recent, so far unpublished, data concerning potential mechanisms mediating motor atonia during cataplectic episodes.

Human narcolepsy is generally caused by loss of hypothalamic hypocretin/orexin neurons (Peyron et al., 2000; Thannickal et al., 2000). Mutations of the hypocretin receptor-2 in dogs also elicits a narcolepsy/cataplexy phenotype (Lin et al., 1999). In mice, genetic deletion of the either the preprohypocretin gene (Chemelli et al., 1999) or transgenic expression of a toxic protein that selectively kills hypocretin neurons (Hara et al., 2001) also elicits narcoleptic symptoms, including cataplexy. These mice are

therefore useful for identifying the mechanisms underlying cataplexy.

A hypothesis is that motor tone is lost during cataplexy because excitatory noradrenergic drive onto motoneurons is reduced (Wu et al., 1999; McGregor and Siegel, 2010). This theory stems from the fact that cells in the locus coeruleus (LC) abruptly stop firing during cataplectic attacks in narcoleptic dogs. Dr. Siegel's laboratory found that LC cell activity is maximal during wakefulness when muscle tone is high, but LC cell discharge activity terminates at cataplexy onset and resumes when the cataplectic attack ends (Wu et al., 1999). Supporting the idea that LC activity regulates muscle tone is the observation the LC cells terminate their activity during REM sleep episodes when postural tone is minimal or absent (Foote et al., 1983; Takahashi et al., 2010). Anatomical and electrophysiological data also suggest the noradrenergic cells project to and regulate motoneuron behavior and muscle tone. Electrophysiology studies show that chemical and electrical stimulation of LC neurons induces short-latency activation of spinal motoneurons (Fung and Barnes, 1987). In addition, immunohistochemical tracing studies indicate that motoneurons receive dense noradrenergic inputs from the locus subcoeruleus and A5/A7 group cells (Levitt and Moore, 1979; Aldes, 1990; Card et al., 1990). Importantly, trigeminal motoneurons express α_1 -adrenoreceptors, which mediate synaptic excitation (Shao and Sutin, 1991). Activation of α_1 -adrenoreceptors on motoneurons triggers increased muscle tone *in vivo* (Fenik et al., 2005; Chan et al., 2006; Schwarz et al., 2008; Schwarz and Peever, 2010) and heightened motoneuron excitation *in vitro* (Parkis et al., 1995). Even though evidence indicates that loss of noradrenergic drive onto motoneurons contributes to cataplexy (Fenik et al., 2005; Chan et al., 2006), it is unknown if changes in noradrenergic activity around trigeminal motoneurons are indeed responsible for triggering cataplexy. My laboratory has therefore begun using hypocretin knockout mice (Chemelli et al., 1999) – a model of human narcolepsy – to determine if changes in noradrenergic transmission mediate cataplexy. To do this, we used two separate, but complimentary experimental approaches. We either manipulate the noradrenergic system by systemically dosing narcoleptic mice with candidate noradrenergic drugs, or we focally manipulate nor-

adrenergic neurotransmission within the trigeminal motor pool during cataplectic episodes. We focus on the trigeminal motor system because masseter muscle tone is routinely lost during cataplexy in both narcoleptic mice and humans (Guilleminault et al., 1974; Burgess et al., 2010). The data discussed below are unpublished and are, therefore, only discussed in general terms.

Noradrenergic system modulates cataplexy in narcoleptic mice

Drugs that affect noradrenergic transmission (e.g., chlomidipramine) are one of the best treatments for human cataplexy (Nishino and Mignot, 1997; Mignot and Nishino, 2005). Such drugs also influence cataplexy in narcoleptic dogs (Mignot et al., 1988). Recent work shows that cataplexy is affected by manipulation of noradrenergic activity in narcoleptic mice (Willie et al., 2003). We also have unpublished data showing that systemic administration of noradrenergic agonists (e.g., phenylephrine) decreases cataplexy. We also observed that noradrenergic antagonists (e.g. terazosin) increase amounts of cataplexy in narcoleptic mice. Together such results support the idea that manipulation of noradrenergic transmission influences murine cataplexy.

Noradrenaline release at both cranial and spinal motor pools is reduced when muscle tone is lost during REM-like sleep (Lai et al., 2001), suggesting that loss of an excitatory drive could underlie motor atonia. Using approaches similar to those described in section 3a (Brooks and Peever, 2008b), we pharmacologically increased noradrenergic tone at trigeminal motoneurons during cataplectic episodes. We found that activation of α_1 -adrenoreceptors increased masseter muscle tone during individual cataplectic episodes (Burgess and Peever, 2009). In fact, masseter tone remained at waking levels during cataplectic attacks, suggesting that loss of an excitatory noradrenergic drive triggers motor atonia during cataplexy and restoring this drive reverses cataplexy.

Multiple studies show that muscle tone is reduced during sleep because an excitatory noradrenergic drive at cranial motor pools is reduced (Fenik et al., 2005; Chan et al., 2006; Mir et al., 2006). Likewise, our recent but unpublished work suggests that the atonia of cataplexy is also triggered, at least in part, by loss of a waking noradrenergic excitation

of trigeminal motoneurons (Burgess and Peever, 2009). In narcoleptic mice, we found that activation of α_1 -adrenergic receptors at the trigeminal motor pool increased waking levels of masseter muscle tone. We also found that blockade of α_1 -adrenoreceptors on trigeminal motoneurons had no effect on masseter EMG tone during individual cataplectic attacks (Burgess and Peever, 2009). Together, these observations suggest that noradrenergic excitation of motoneurons triggers muscle tone during waking and loss of this drive is, at least in part, responsible for suppression of motor atonia during cataplexy. Because blocking α_1 -adrenoreceptors did not reduce waking tone to cataplectic levels, this suggests that other transmitters also function to reduce motoneuron activity during cataplexy. Evidence indicates that the dopamine system is also an important player in the regulation of cataplexy (Burgess et al., 2010). Determining the transmitters that reduce motoneuron activity during cataplexy will undoubtedly provide insight into identifying the potential brain circuits responsible for triggering cataplectic attacks.

Summary

Current data refutes the long-standing hypothesis that glycinergic inhibition of motoneurons is the only mechanism mediating REM atonia. However, we show that multiple inhibitory mechanisms are responsible for triggering loss of trigeminal motoneuron activity and muscle tone during REM sleep. Although inhibition of trigeminal motoneurons during REM sleep generates atonia, neither glycine nor GABA_A receptors themselves are directly responsible. Instead, we have unpublished, preliminary data suggesting that both metabotropic GABA_B and ionotropic GABA_A/glycine receptor-mediated inhibition of trigeminal motoneurons is required for causing REM atonia. Transgenic mice with deficient glycine and GABA transmission have a behavioral, motor and sleep phenotype that recapitulates the cardinal features of RBD. Specifically, mice exhibit REM motor behaviors, heightened phasic REM twitch activity, NREM myoclonus, sleep disruption and EEG slowing. However, levels of REM atonia remain completely intact. Importantly, the RBD phenotype is rescued by drugs (e.g., clonazepam) that treat human disease symptoms. These findings are the first to identify a

potential genetic mechanism for RBD. I propose that these mice are a useful resource for investigating *in-vivo* disease mechanisms and developing potential therapeutics for RBD.

Motor atonia is common to both cataplexy and REM sleep. Our current unpublished findings suggest that REM atonia is triggered by motoneuron inhibition, whereas, cataplexy is primarily caused by reduced motoneuron excitation. We suggest that loss of a waking noradrenergic drive onto trigeminal motoneurons is partially responsible for triggering the atonia of cataplexy in narcoleptic mice. Pharmacological restoration of this noradrenergic drive by direct motoneuron activation with noradrenaline or systemic increases in noradrenergic tone both function to mitigate the atonia of cataplexy. However, evidence also indicates that additional neurotransmitters, such as dopamine, also function to control and modulate cataplexy.

Acknowledgements

The Canadian Institutes of Health Research and the National Science and Engineering Research Council of Canada provided research funding. I thank Patti Brooks, Christian Burgess, Peter Schwarz, Nicole Yee, Gavin Tse, Lauren Gillis and Ben Johnson for their hard work and dedication to sleep science.

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