# Dynamic interactions of excitatory and inhibitory inputs in hypoglossal motoneurones: respiratory phasing and modulation by PKA

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**The balance of excitation and inhibition converging upon a neurone is a principal determinant of neuronal output. We investigated the role of inhibition in shaping and gating inspiratory drive to hypoglossal (XII) motoneuronal activity. In neonatal rat medullary slices that generate a spontaneous respiratory rhythm, patch-clamp recordings were made from XII motoneurones, which were divided into three populations according to their inhibitory inputs: non-inhibited, inspiratory-inhibited and late-inspiratory-inhibited. In late-inspiratory-inhibited motoneurones, blockade of $GABA_A$ receptors with bicuculline abolished inspiratory-phased inhibition and increased the duration of inspiratory drive currents. In inspiratory-inhibited motoneurones, bicuculline abolished phasic inhibition, which frequently revealed excitatory inspiratory drive currents. In non-inhibited motoneurones, neither bicuculline nor strychnine markedly changed inspiratory drive currents. Inhibitory currents in XII motoneurones were potentiated by protein kinase A (PKA) activity. Intracellular dialysis of the catalytic subunit of PKA or bath application of the PKA activator Sp-cAMP significantly increased the amplitude of expiratory-phased IPSCs without any change in IPSP frequency. Inspiratory-phased inhibition in inspiratory-inhibited motoneurones was potentiated by Sp-cAMP. We conclude that inspiratory-phased inhibition is prevalent in neonatal XII motoneurones and plays an important role in shaping motoneuronal output. These inhibitory inputs are modulated by PKA, which also modulates excitatory inputs.**

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Motoneurones integrate excitatory and inhibitory inputs to produce motor output and consequent behaviour (Kirkwood *et al.* 1993; Rekling *et al.* 2000). Postsynaptic receptors mediating excitatory inputs to motoneurones are dynamically controlled by protein kinases and phosphatases (Bocchiaro *et al.* 2003); whether there are similar effects on behaviourally relevant inhibitory currents is not known.

In the neonatal rodent medullary slice preparation, respiratory-related motor output persists after blockade of both glycine and $GABA_A$ receptors (Smith *et al.* 1991; Shao & Feldman, 1997) indicating that inhibition is not required for respiratory rhythm generation (in this preparation). Although noted *in vivo* (Withington-Wray *et al.* 1988; Woch & Kubin, 1995), the role of respiratory-modulated inhibition in functionally active XII motoneurones is unclear, as detailed cellular studies of XII motoneuronal inhibition have only been performed in non-rhythmically active preparations (O'Brien & Berger, 1999; Donato & Nistri, 2000, 2001; Nistri & Donato, 2001). Hence, the functional and behavioural significance of inhibition on respiratory-related XII output is unknown.

XII motoneurones innervate the tongue and upper airway muscles (Dobbins & Feldman, 1995; Veasey, 2003). The control of XII motoneuronal excitability is of clinical interest, as pathophysiological changes may underlie upper airway collapse causing snoring or obstructive sleep apneoa (OSA). Changes in inhibition of XII motoneurones may contribute to OSA. Alcohol (Taasan *et al.* 1981; Issa & Sullivan, 1982; Scrima *et al.* 1982) and REM sleep (Weigand *et al.* 1991) increase the incidence and severity of OSA, both of which are associated with increased inhibition in XII motoneurones (Yamuy *et al.* 1999). In humans (Krol *et al.* 1984) and rats (Gibson & Berger, 2000) alcohol depresses XII nerve but not phrenic nerve (Krol *et al.* 1984; DiPasquale *et al.* 1995) activity

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suggesting XII motoneurones are specifically susceptible to ethanol. Ethanol potentiates glycinergic mIPSCs in XII motoneurones (Eggers *et al.* 2000); hence, the depressant effects of ethanol on XII motoneurones (DiPasquale *et al.* 1995; Gibson & Berger, 2000) could be mediated *via* facilitation of both glycinergic and GABAergic inhibition.

The effects of inhibition on XII motoneuronal excitability may be modulated by protein kinases. For example, PKA modulates GABAergic currents (Moss *et al.* 1992; McDonald *et al.* 1998; Fancsik *et al.* 2000; Jung *et al.* 2000). PKA is abundantly expressed in XII motoneurones (S. Saywell and J. L. Feldman, unpublished observations), whereas, except for protein kinase G (PKG) (DeVente *et al.* 2001), other protein kinases appear absent or expressed at low levels (Erondu & Kennedy, 1985; Saito *et al.* 1988; Ito *et al.* 1990; Nakamura *et al.* 1995; Nakamura *et al.* 1996; Naik *et al.* 2000). These data provoked us to investigate if PKA can also modulate inhibitory inputs in XII motoneurones.

Here, we characterize inspiratory-modulated inhibitory inputs to XII motoneurones and demonstrate that these inputs are affected by PKA. We propose that these inhibitory inputs are pivotal in controlling XII motoneuronal excitability and that pathophysiological changes in PKA activity in XII motoneurones may contribute to OSA.

## Methods

### Slice preparation

All animal experiments were performed with the approval of the UCLA Institutional Animal Care and Use Committee.

Experiments were performed on neonatal Sprague–Dawley rats (P0–P4) anaesthetized by hypothermia for a minimum of 3 min, and upon the absence of limb withdrawal to noxious pinch, they were rapidly decerebrated. A medullary slice preparation was prepared that retains a sufficient proportion of the respiratory network to generate a respiratory related rhythm (Smith *et al.* 1991). Briefly, the brainstem and upper cervical cord was isolated and bathed in artificial cerebrospinal fluid (ACSF) of composition (mM): NaCl 128.0, KCl 3.0, $CaCl_2$ 1.5, $MgCl_2$ 1.0, $NaHCO_3$ 23.5, $NaH_2PO_4$ 0.5, D-glucose 30.0, pH 7.4, bubbled with 95% $O_2$–5% $CO_2$ pH 7.4 at room temperature, the dura, superficial blood vessels and the cerebellum were then removed. The brainstem was then mounted on a chuck and serial transverse sections (200–300 $\mu$m) were cut with a Vibratome (Technical Products International, VT 100) until the identifiable landmarks of compact formation of the nucleus ambiguous and the inferior olive could be seen; a transverse 700 $\mu$m slice including the pre-Bötzinger complex (preBötC) was then cut. The slice was transferred to a recording chamber and superfused (5 ml $min^{-1}$) with ACSF containing elevated $K^+$ (9 mM) to induce a stable respiratory-related output. The slice was maintained at a constant temperature of 28°C.

### Hypoglossal nerve (XIIn) recording

A suction electrode was applied to the cut ends of the XII nerve (XIIn) rootlets, and discharges from the XIIn recorded, amplified 5000× and filtered at 1 kHz using a preamplifier (Grass). Population discharges of the XIIn rootlets were then rectified and integrated using a leaky integrator ($\tau = 100$ ms). Signals were digitized and stored on hard disk using pCLAMP software (Axon Instruments). The discharges of the XII nerve were used to define the inspiratory period.

### Patch-clamp recording

XII motoneurones were visualized using IR-DIC microscopy; neurones were considered to be XII motoneurones according to the criteria of Funk *et al.* (1993). Whole-cell voltage-clamp recordings (holding potential $V_h = -70$ mV) were made from XII motoneurones using electrodes pulled from borosilicate glass on an electrode puller (Sutter Instruments, Model P-87), and filled with patch solution of the composition (mM): 120 potassium gluconate, 11 glycol-bis-(b-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA), 5 NaCl, 1 $CaCl_2$, 10 Hepes, 2 ATP ($Mg^{2+}$ salt), pH 7.3 adjusted with KOH (resistance 4–8 MΩ). To help confirm the cells as motoneurones, Lucifer Yellow (Molecular Probes) was included in the patch solution to intracellularly label the neurones. Neurones were subsequently examined under an epiflourecence microscope (Axioskop, Zeiss) to confirm their location, examine their morphology and identify axons projecting in the XIIn tract. The patch-clamp electrode was advanced toward neurones under positive pressure. Once the electrode tip approached the cell, positive pressure was released and a gigaohm seal formed by negative pressure application. Neurones were then ruptured by application of brief negative pressure. Access resistance was continually monitored and was always less than 30 MΩ. Cells with large or unstable access resistances were rejected for analysis. Intracellular signals were acquired using an Axopatch

1D amplifier, filtered using a –3 dB Bessel filter and digitized at 10 kHz *via* a Digidata 1200 interface with a software filter (bandpass: 2 Hz–5 kHz) in pCLAMP software (Axon Instruments). Junction potentials between bath solution and electrode were corrected for and whole-cell capacitance was compensated. Chloride-mediated currents were tested for by reversal ($V_h = -100$ mV).

### Averaging and data analysis

Averages of respiratory-related membrane currents were constructed using the rising phase of the integrated XIIn activity to trigger acquisition of a 5 s epoch of membrane current. Typically averages of 10 consecutive respiratory cycles were constructed; however, occasionally averages of up to 90 sweeps were constructed. Recordings were analysed off-line using Clampex software (Axon Instruments) and exported to Origin (OriginLab Corp.).

The characteristics of IPSCs occurring during the expiratory period were analysed using Mini-Analysis Program (Synaptosoft). IPSCs were identified according to criteria of rise time and amplitude; currents with a slow rise time or an amplitude of $\leq$ 20 pA were excluded from analysis, and hence only clearly identifiable IPSCs were analysed. Similarly, if there was any evidence of coincident EPSCs the currents were rejected from analysis. Cumulative histograms analysing IPSC amplitude and frequency were constructed and the Kolmogorov–Smirnoff statistical test was used to determine significance for each individual recording pre- and post-drug application; for details see Shao & Feldman (2001). Student's *t* test was used to determine statistical significance for all other data. Statistical significance was assessed as a $P \leq 0.05$; results are given as means ± S.D.

### Drugs and drug application

Drugs were either bath applied (bicuculline (10 $\mu$M), strychnine (1 $\mu$M), Sp-adenosine 3′,5′-cyclic monophosphorothioate triethylammonium salt (Sp-cAMP: 100 $\mu$M)) or included in the recording electrode for intracellular dialysis (catalytic subunit of PKA; cPKA: 250 units ml$^{-1}$). All drugs were obtained from Sigma.

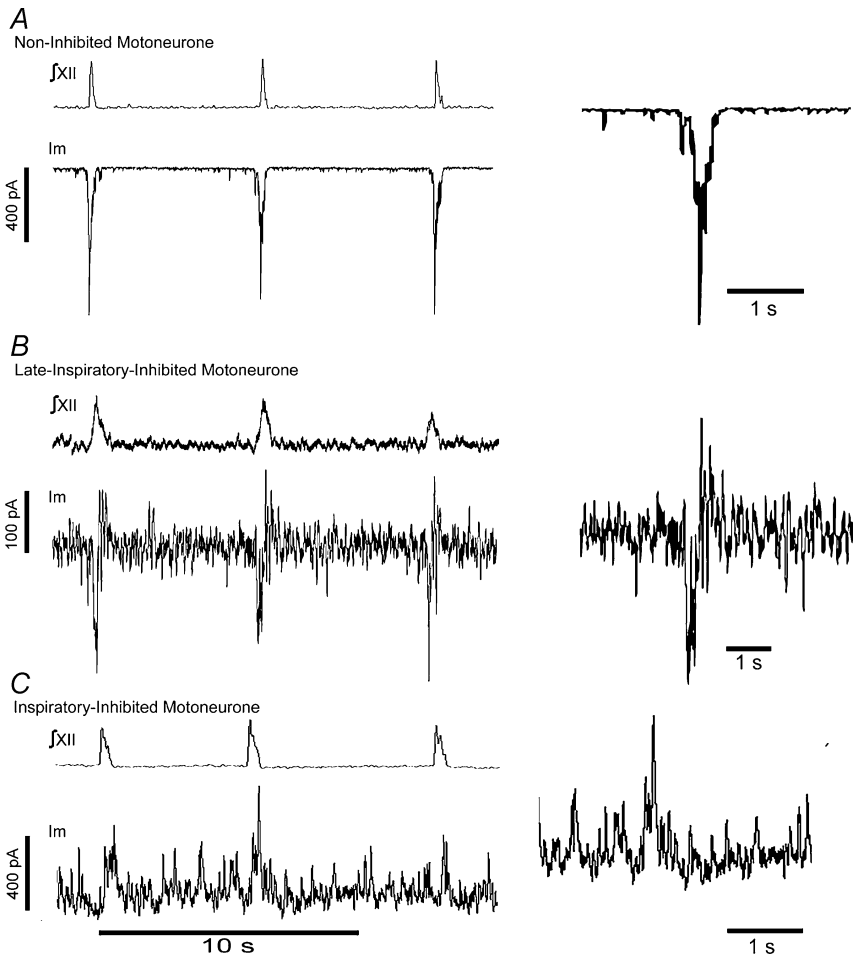


**Figure 1. Examples of the three populations of motoneurones**
*A–C*, examples of intracellular recordings from motoneurones. Upper traces (∫XII), integrated discharge of the XII nerve; lower trace (Im) voltage-clamp recordings from XII motoneurones. *A*, a non-inhibited motoneurone; note the absence of positive-going inhibitory currents. *B*, a late-inspiratory-inhibited motoneurone; note the predominance of upward inhibitory currents toward the latter portion of the inspiratory period as defined by the duration of the integrated XII nerve discharge. *C*, an inspiratory-inhibited motoneurone; note the positive-going inhibitory currents in phase with the inspiratory period as defined by the XII nerve discharge. Inset on right shows an example of an individual respiratory cycle.

## Results

### Motoneurone recordings and identification

Patch-clamp recordings were made from visualized XII motoneurones ($n = 33$) in a region containing motoneurones that innervate the genioglossus muscle of the tongue (Nunez-Abades *et al.* 1994). All neurones conformed to the criteria of Funk *et al.* (1993) for identifying XII motoneurones and were characteristic of neurones described in that study. Motoneurones filled with Lucifer Yellow had large ($> 15$ $\mu$m) multipolar somata, frequently with axons projecting into the XIIn tract. Most inspiratory-modulated motoneurones ($n = 30/33$) displayed clear outward, i.e. inhibitory, postsynaptic currents (IPSCs). IPSCs could be reversed within 60–100 s after whole-cell patch formation by changing the holding potential from −70 mV to −100 mV. Motoneurones were divided into three populations according to the level of inspiratory-phased inhibitory drive (Fig. 1): non-inhibited (no inspiratory phased inhibition; 3 motoneurones had some inhibition during the expiratory period, c/w Fig. 3), late-inspiratory-inhibited (inhibition in the later half of the inspiratory period) and inspiratory-inhibited (inhibition throughout inspiration).

### Late-inspiratory-inhibited motoneurones: GABAergic inhibition truncates excitatory drive currents

Late-inspiratory-inhibited motoneurones ($n = 15/33$) received phasic inhibition after the peak of XII nerve activity that continued into the postinspiratory period (Figs 1*B* and 2). Usually, late-inspiratory inhibition could be easily identified in averaged traces (Fig. 2*B*).

To determine the pharmacology of the postsynaptic inhibition identified by changing the holding potential to –100 mV, bicuculline (10 $\mu$M: $n = 9$) was bath applied to block $GABA_A$ inputs. Bicuculline abolished late-inspiratory inhibition and increased the duration of inspiratory drive currents, with negligible effects on peak amplitude (Figs 2*C* and 3). Bicuculline abolished all but a small proportion of unitary inward, i.e. inhibitory, currents in late-inspiration and completely abolished inhibitory inputs in the postinspiratory period (Fig. 3*C*). Changing the holding potential to −100 mV caused the unaffected currents to reverse (Fig. 3*B* and *C*), suggesting that they are mediated via $GABA_C$ or glycine receptors.

To assess the effect of late-inspiratory inhibition upon the pattern of XII motoneuronal inspiratory drive currents, late-inspiratory-inhibited motoneurones ($n = 6$) were compared to non-inhibited motoneurones ($n = 6$), e.g. Fig. 1*A*. Timing of events was computed relative to the beginning and end of concurrent XIIn inspiratory bursts, which were considered to define the inspiratory period. In order to fairly compare the relative timing, all data were normalized to the duration of the XII burst by calculating the occurrence of the event as a percentage of the inspiratory period, with negative values indicating events preceding the onset of bursting and values greater than 1 indicating occurrence after burst termination.

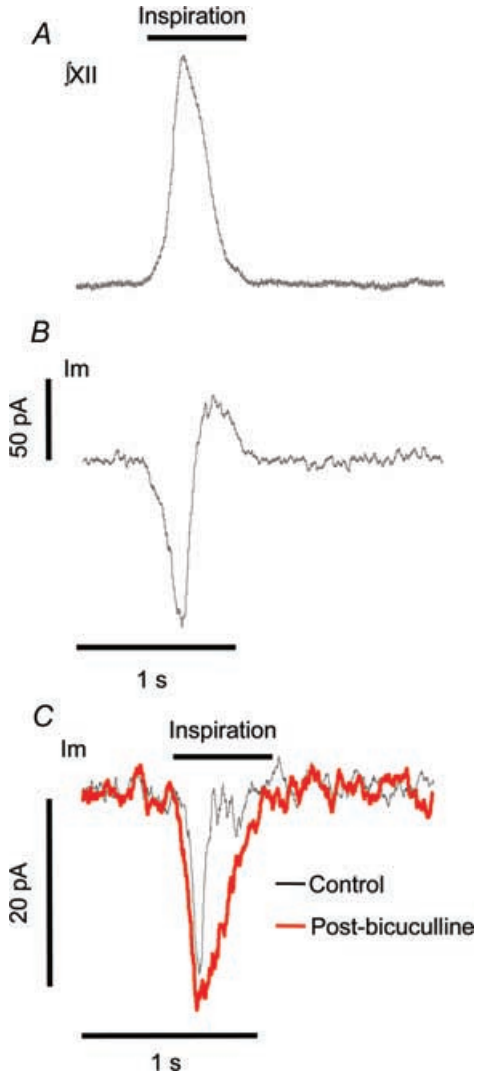


**Figure 2. Phasic inhibition during the late inspiratory period**
*A*, upper trace, ∫XII nerve discharge (90 sweeps). *B*, average of voltage-clamp recordings ($V_h = -70$ mV) from a late-inspiratory-inhibited motoneurone illustrating the extensive inhibition occurring in the second half of the Inspiratory period (90 sweeps). *C*, inspiratory drive currents pre- and postbicuculline application (10 $\mu$M) from a different late-inspiratory-inhibited motoneurone ($V_h = -70$ mV). Bicuculline has negligible effect on the amplitude of the drive current but markedly increased its duration.

For both late-inspiratory-inhibited and non-inhibited motoneurones, inspiratory drive currents began before the onset of XII nerve activity (Fig. 4). The relative onset of outward, i.e. excitatory, currents in late-inspiratory-inhibited motoneurones (−14 ± 7%) was significantly earlier than for non-inhibited motoneurones (−6.3 ± 3.3%; $P < 0.01$). There were no significant differences in the timing of their peak currents ($P > 0.05$; Fig. 4). The duration of the excitatory inspiratory drive currents were significantly shorter in late-inspiratory-inhibited motoneurones (56 ± 28%) compared to those in non-inhibited motoneurones (101 ± 5%, $P < 0.01$, $n = 6$; Fig. 4).

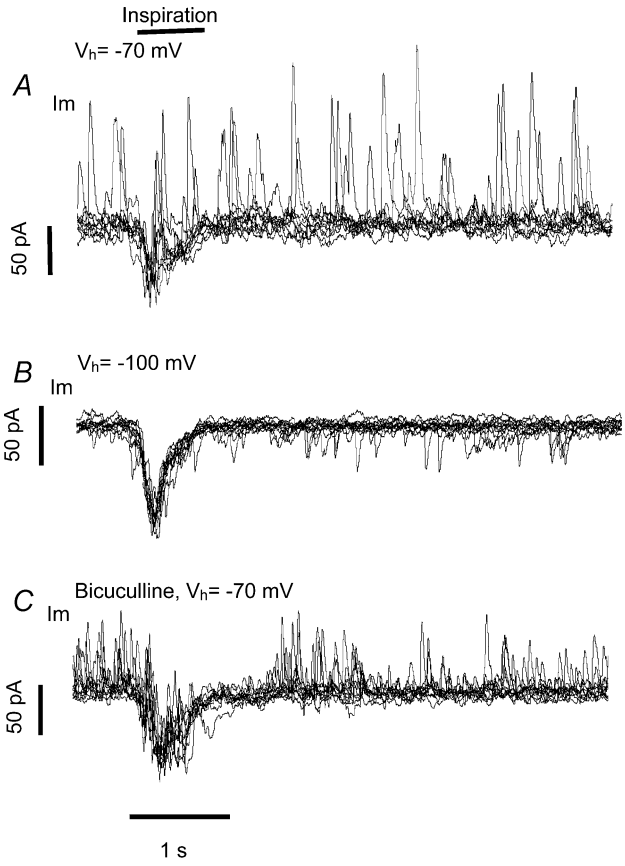


**Figure 3. Bicuculline-sensitive inhibition during inspiration and the postinspiratory period**
*A*, overlays of 10 consecutive voltage-clamp recordings from a late-inspiratory-inhibited motoneurone that received strong inhibition throughout all phases of the respiratory cycle. IPSCs are positive-going deflections. *B*, reversal of the IPSCs by changing the holding potential to –100 mV (10 consecutive recordings). Note that the current characteristics now resemble those of a non-inhibited motoneurone. IPSCs become negative going. *C*, application of bicuculline (10 $\mu$M) reduced the number of IPSCs during the inspiratory period and abolished IPSCs during the postinspiratory period (10 consecutive recordings). Note that although reduced in amplitude IPSCs persist during the expiratory period indicating respiratory phased inhibition.

### Inspiratory-inhibited motoneurones: blockade of GABAergic inhibition reveals excitatory drive currents

For inspiratory-inhibited motoneurones, e.g. Fig. 1*C* ($n = 6$), inhibitory currents peaked coincidently with peak XII nerve activity and in general paralleled typical XIIn discharge (Figs 5 and 7). Inspiratory-phased inhibition rapidly peaked and then decremented (Fig. 5*A*), similar to the pattern of excitatory drive currents (Figs 1*A* and 3). Bicuculline completely abolished all inspiratory-phased inhibitory inputs (Figs 5 and 7) and revealed excitatory inspiratory drive currents in 50% of inspiratory-inhibited motoneurones ($n = 3$; Figs 5*B* and 7). Occasionally clear inspiratory-phased inhibition was seen as an increased frequency of IPSCs during the inspiratory period (Fig. 5*B*), without any identifiable positive-going waveform, e.g. Fig. 5*A*. Annihilation of excitatory and inhibitory inputs could explain the absence of an obvious outward current in a small proportion of late inspiratory-inhibited

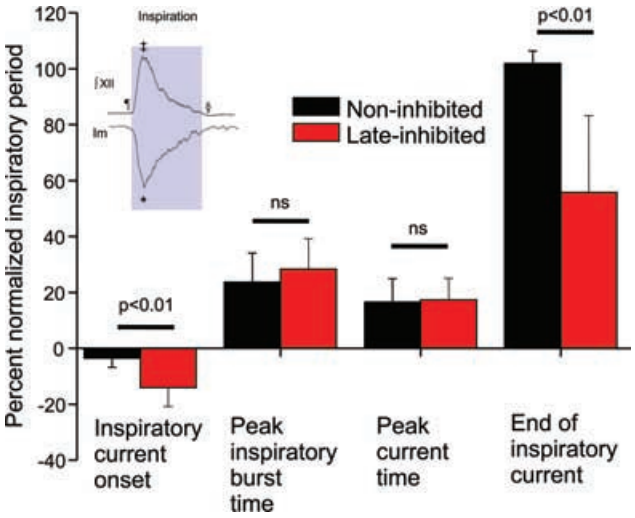


**Figure 4. Comparison of the characteristics of non-inhibited and late inspiratory-inhibited motoneurones**
Bar charts representing the respective timing of various events within the inspiratory period. The inspiratory period for 6 late-inspiratory-inhibited motoneurones and 6 non-inhibited motoneurones was normalized to the duration of inspiration as defined by the XII nerve activity. Inset: a typical recording from a non-inhibited motoneurone; upper trace, ∫XIIn discharge (∫XII); lower trace, inspiratory current (Im), illustrating the duration of inspiration (shaded area). The time of occurrence of onset of inspiratory current (¶), time of peak integrated XII nerve discharge (‡), time of peak inspiratory current (∗) and termination of inspiratory drive currents (§) were measured and plotted as the percentage time of occurrence in the inspiratory cycle. Negative values indicate events occurring before the onset of the inspiratory burst. Statistical significance was assessed using unpaired *t* tests and a value of $P \leq 0.05$ was considered significant; ns, not significant.

motoneurones (Fig. 2*C*) despite convincing inhibition in this period as demonstrated pharmacologically.

### Expiratory phased IPSCs are potentiated postsynaptically by PKA

To investigate if endogenously active IPSCs were modulated by PKA, the activated catalytic subunit of PKA (cPKA; 250 units $ml^{-1}$) was included in the patch-clamp electrode. Expiratory-phased IPSCs were analysed for amplitude and frequency. IPSCs occurring in the initial period of the recording shortly after patch formation (within 4 min) were compared to IPSCs after 20 min, a period sufficient for the catalytic subunit to dialyse the cell (Bocchiaro *et al.* 2003). For each recording, dialysis of cPKA significantly potentiated IPSC amplitude from $115 \pm 106$ to $141 \pm 246$ pA ($P < 0.001$; Kolmogorov–Smirnoff test); however, there was no significant change in IPSC frequency ($n = 7$; Fig. 6).

To investigate if endogenous PKA activity potentiated IPSC amplitude, Sp-cAMP (100 $\mu$M), a non-hydrolysable cell-permeant cAMP analogue, was bath applied. Sp-cAMP significantly potentiated the amplitude of expiratory phased IPSCs 20 min after patch formation, from $35 \pm 10$ to $43 \pm 17$ pA ($P < 0.01$; $n = 4$) without significant changes in their frequency (Fig. 6).

### Inspiratory-phased GABAergic inhibition is potentiated by activation of PKA

To investigate if inspiratory phased GABAergic inhibition was similarly potentiated by activation of PKA, Sp-cAMP (100 $\mu$M) was bath applied. The amplitude of inspiratory phased inhibitory currents were potentiated by PKA activation, as was the overall inhibitory charge transfer $148 \pm 10\%$ ($n = 4$; $P = 0.001$) (Fig. 7). Application of bicuculline could still antagonize inhibitory currents during inspiration in inspiratory-inhibited motoneurones

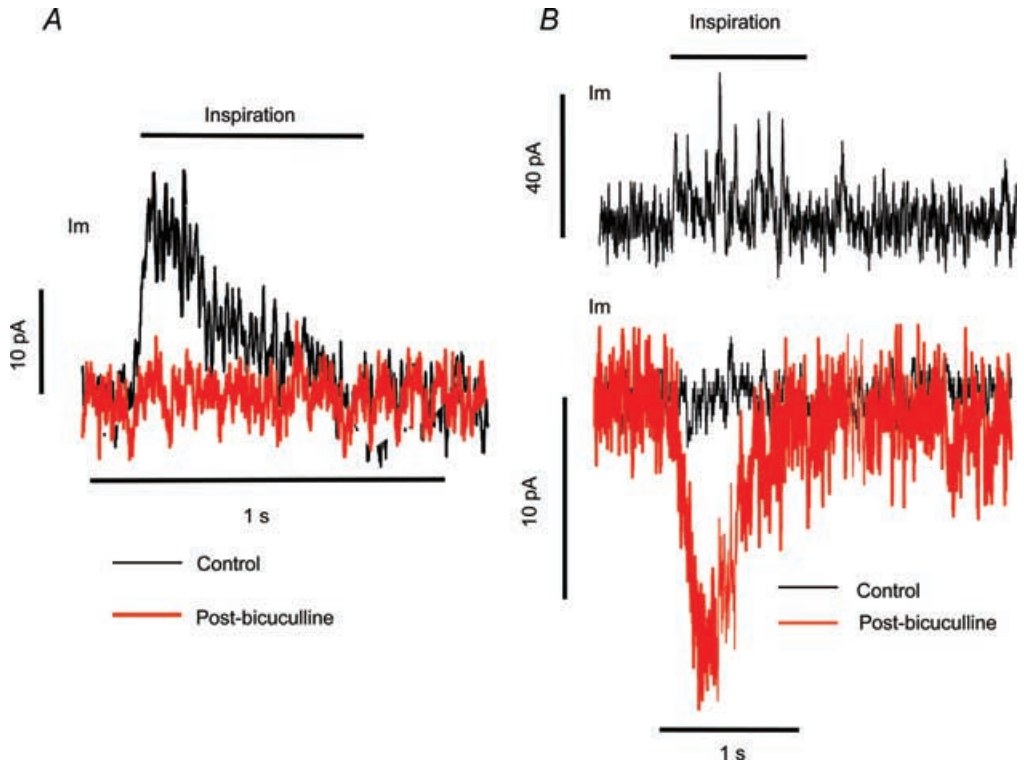


**Figure 5. Inspiratory phased inhibition occludes excitatory postsynaptic currents**
*A*, voltage-clamp recordings of inspiratory phased IPSCs in an inspiratory-inhibited motoneurone (30 sweeps). Averages show a clear inspiratory phased inhibition (Control) that is abolished by application of bicuculline (10 $\mu$M). *B*, voltage-clamp recording of inspiratory phased IPSCs in a second inspiratory-inhibited motoneurone. Upper trace, inspiratory triggered acquisitions of membrane current showing inspiratory phased IPSCs. Lower trace, inspiratory triggered averages (Control) failed to reveal inhibitory currents (100 sweeps). Despite the increased incidence of IPSCs during the inspiratory period (top trace) there is no apparent synchrony of events within the inspiratory period precluding averaging. Bicuculline application (10 $\mu$M) revealed an excitatory inspiratory drive current (30 sweeps).

($n = 4$), and occasionally revealed a weak excitatory drive (Figs 5*B* and 7). Further application of strychnine (1 $\mu$M) had no additional effects upon the size of the revealed excitatory drive (Fig. 7, $n = 4$). Bath application of Sp-cAMP produced no increase in integrated XIIn activity, indicating there is no overall change in the balance of excitation and inhibition due to the activation of PKA.

## Discussion

The balance and relative strengths of inhibitory and excitatory inputs in motoneurones is a principal determinant of motor output, which produces movement and behaviour. Concurrent respiratory-phased excitation and inhibition in motoneurones is present *in vivo* (Withington-Wray *et al.* 1988; Woch & Kubin, 1995) and

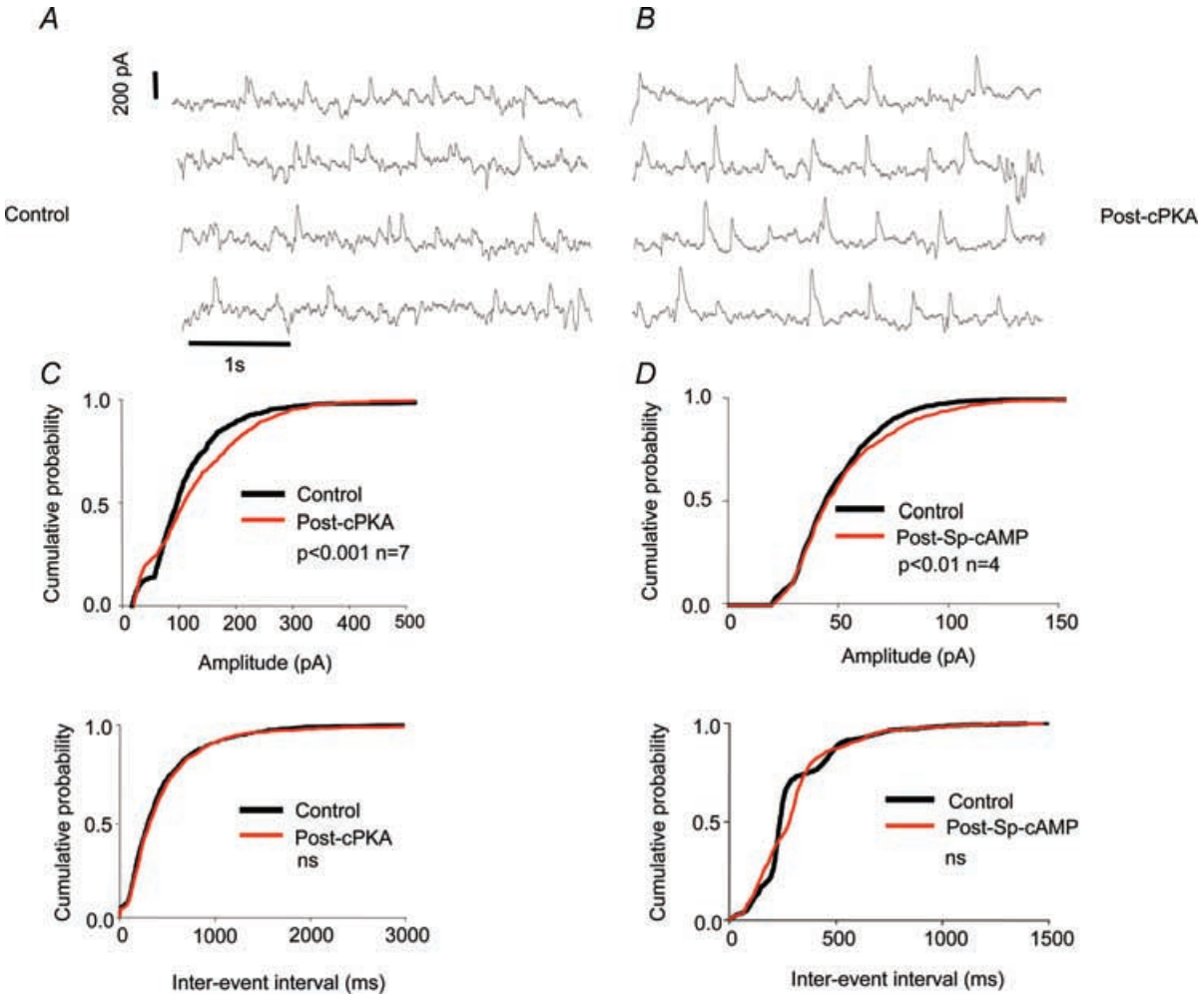


**Figure 6. PKA potentiated expiratory phased IPSCs via a postsynaptic mechanism**
*A*, expiratory IPSCs recorded within the first few minutes of patch formation. Note that these currents are clearly outward. *B*, expiratory IPSCs recorded from the same neurone 20 min after patch-formation with cPKA (250 units $ml^{-1}$) included within the patch-clamp electrode. *C*, upper histogram, cumulative histogram of IPSC amplitude pre- and postdialysis of the motoneurone with cPKA; a rightward shift in the curves indicates a statistically significant ($P < 0.01$) potentiation of the IPSCs. Lower histogram, cumulative histogram of the interevent interval. No statistical difference in the curves indicates that the effects are limited to the postsynaptic site. *D*, upper histogram, cumulative histogram of IPSC amplitude pre- and postapplication of Sp-cAMP (100 $\mu$M). A rightward shift in the curves indicates a statistically significant ($P < 0.01$) potentiation of the IPSCs. Lower histogram, cumulative histogram of the interevent interval; non-significant change in the curves indicates that the effects are limited to the postsynaptic site.

*in vitro* (Parkis *et al.* 1999). However, pharmacologically studying identified neurones in *in vivo* preparations are challenging (Richter *et al.* 1997) and not readily amenable to stable recordings allowing manipulation of intracellular second messengers (Lalley *et al.* 1997). Endogenously rhythmic respiratory *in vitro* preparations are more amenable to such studies, particularly in the context of behaviour.

We observed phasic inhibition in XII motoneurones receiving inspiratory drive in slices from neonatal rats and have shown that (i) there are multiple species of respiratory-phased inhibitory inputs; (ii) late-inspiratory GABAergic inhibition can truncate excitatory inspiratory drive currents; (iii) inspiratory-phased GABAergic inhibition is coincident with inspiratory-phased excitation and can occlude XII motoneuronal activity; and (iv) inspiratory and expiratory phased inhibitory inputs to XII motoneurones are modulated by PKA.

We defined three subpopulations of XII motoneurones according to their balance of excitation and inhibition.

**1** Non-inhibited motoneurones that receive strong excitatory inspiratory drive currents (Funk *et al.* 1993; Funk *et al.* 1995; Robinson *et al.* 2002; Bocchiaro *et al.* 2003) with little concurrent inhibition. Changes in their membrane potential to values that would reverse $Cl^-$ (or $K^+$) currents, or application of antagonists to inhibitory receptors, had little effect on their inspiratory-modulated currents. Inhibition was often evident in the expiratory period (c/w Fig. 3).

**2** Late-inspiratory-inhibited motoneurones received inspiratory phased excitatory drive coupled with an inhibitory input during late-inspiration. Inhibition typically began after peak XII nerve discharge and continued into the postinspiratory period. However, occasionally the inhibition could not be identified in the average although evident in raw traces and revealed by reversal of the membrane potential and pharmacological blockade. Blockade of $GABA_A$ receptors with bicuculline eliminated late-inspiratory phased inhibition without abolishing expiratory phased inhibition, suggesting it does not include a glycinergic component (Jonas *et al.* 1998; O'Brien & Berger, 1999). This inhibition is presumably mediated *via* inhibitory GABAergic premotoneurones that receive excitatory inspiratory drive. These premotoneurones have a similar activity to those seen in *in vivo* preparations in the region of the nucleus of the solitary tract (NTS) (Cohen & Feldman, 1984; Cohen *et al.* 1993) and ventral respiratory column

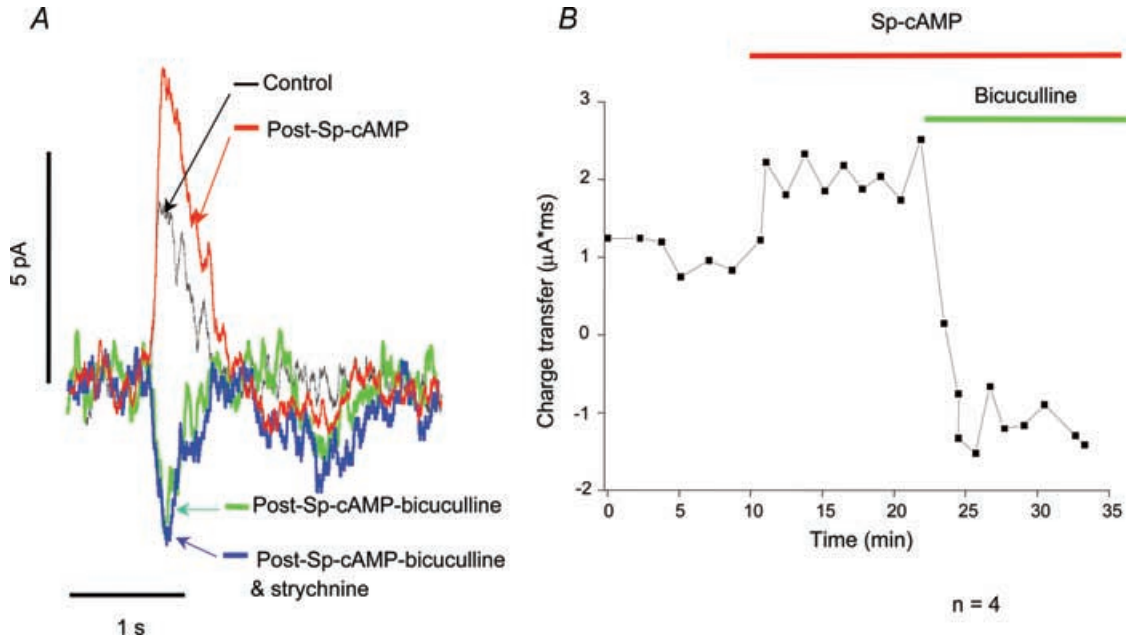


**Figure 7. PKA potentiated inspiratory phased $GABA_A$ currents**
*A*, voltage-clamp recordings from an inspiratory-inhibited motoneurone. Averages of membrane current revealed inspiratory phased inhibition (Control, 70 sweeps). Activation of PKA by Sp-cAMP (100 $\mu$M) potentiated inspiratory phased inhibition (Post-Sp-cAMP, 80 sweeps). Bicuculline (10 $\mu$M) blocked this inhibition and revealed excitatory drive currents (Post-Sp-cAMP-bicuculline, 45 sweeps); subsequent blockade of glycinergic inhibition with strychnine (1 $\mu$M) has no further effect (Post-Sp-cAMP-bicuculline & strychnine, 20 sweeps) ($n = 4$). *B*, graph of changes in charge transfer due to activation of PKA by Sp-cAMP and blockade of inhibition with bicuculline.

(Oku *et al.* 1992; Cohen *et al.* 1993). The location of these premotoneurones in the slice preparation is unknown.

Late-inspiratory inhibition clearly truncates inspiratory drive currents, indicating that phasic inhibition can terminate motoneurone discharge at the transition from inspiration to expiration, even though inspiratory XII motor activity can continue through the postinspiratory period. Thus, the transition from inspiration to expiration is not only controlled at the level of the premotor interneurones (Ballantyne & Richter, 1984) but also at the level of the motoneurone.

**3** Inspiratory-inhibited motoneurones received inhibition throughout the inspiratory period. Inhibition typically began at the onset of inspiration and peaked coincidently with peak XII nerve discharge. Since bicuculline abolished inhibition in these motoneurones, this inhibition is mediated via inspiratory-phased GABAergic interneurones. This is in contrast to the pharmacology of inspiratory phased interneurones that inhibit preBötC neurones, which are glycinergic (Shao & Feldman, 1997). Blockade of $GABA_A$ receptor-mediated inhibition in inspiratory-inhibited motoneurones frequently revealed inspiratory-phased excitatory drive currents, indicating that motoneurones are actively gated out by phasic inhibition that effectively modulates motoneurone recruitment. Bicuculline application may cause disinhibition of premotoneurones mediating the excitatory drive and resulting in increased input to motoneurones. However, blockade of inhibition in non-inhibited motoneurones had little effect upon the amplitude or duration of their excitatory inspiratory drive currents as would be expected if there was significant premotor disinhibition. This would suggest that it is unlikely the revealed excitatory drive currents are due to disinhibition of premotor neurones. The presence of coincident inspiratory excitatory drive currents revealed by blockade of GABAergic inputs would indicate that this inhibition gates inspiratory-inhibited motoneurones. In this regard, decreasing this inhibition would be beneficial when increased muscle tone is needed in response to changing physiological demands or during behaviours like gasping when activation of greater numbers on motoneurones is required. Alternatively, changes in the level of inhibition may be involved in the induction and expression of long-term facilitation (Feldman *et al.* 2003). Excessive inhibition of this type, e.g. during sleep or after alcohol consumption, could prevent motoneuronal recruitment and contribute to OSA.

AMPA receptor-mediated inspiratory drive currents to XII motoneuronal excitability are modulated by PKA and protein phosphatases (Bocchiaro *et al.* 2003). Here we found that inhibitory currents were similarly modulated by PKA. Expiratory phased IPSCs were significantly potentiated by intracellular dialysis of cPKA, indicating that inhibitory receptors or associated proteins were substrates for PKA. Similarly, activation of native PKA by Sp-cAMP significantly potentiated expiratory phased IPSCs. As Sp-cAMP is cell-permeant, bath-application will activate PKA at both pre- and postsynaptic sites. Presynaptically, PKA may act to increase transmitter release or preterminal excitability and consequently potentiate inhibitory inputs. This does not appear to be the case, as the frequency of individual IPSCs did not change; PKA presumably potentiated inhibition postsynaptically.

Activation of PKA with Sp-cAMP markedly potentiated GABAergic inhibition in inspiratory-inhibited motoneurones indicating that PKA is important in modulating the gating of XII motoneurones. Activation of PKA caused no changes in the amplitude or duration of the integrated XII nerve burst, indicating that PKA did not shift the balance of excitatory and inhibitory drives. Although activation of PKA potentiates $GABA_A$ receptor-mediated currents, we have not determined the pharmacology of expiratory phased IPSCs potentiated by PKA. From the IPSC characteristics it appears that both GABAergic and glycinergic currents are present (O'Brien & Berger, 1999).

We conclude that phasic inhibition is pivotal in controlling XII motoneuronal excitability and, *in vitro*, inhibition in XII motoneurones appears much stronger than that observed in phrenic motoneurones (Parkis *et al.* 1999). Hence the role of inhibition in XII motoneurones may be particularly important in the control of XII excitability. Consequently this may confer on these motoneurones a greater sensitivity to changes in inhibition due to alcohol or REM sleep. Decreases in XII motoneuronal excitability during sleep (Weigand *et al.* 1991) contributes to OSA, with clinical studies clearly implicating increased inhibition as contributing to this decreased excitability (Krol *et al.* 1984). Thus, pathological changes in inhibition could contribute to OSA. Increased inhibition of XII motoneurones could be due to many factors including changes in GABA metabolism (Engel *et al.* 2001), modulation of GABA transporters, whose function is affected by protein kinases (Law *et al.* 2000), changes in protein kinase activity (Melis *et al.* 2002), or age-related changes in receptor expression (O'Brien & Berger, 2001). We suggest that the marked effect of alcohol on PKA activity (Melis *et al.* 2002) would lead to postsynaptic potentiation of GABA receptor function in XII motoneurones and consequent exacerbation of OSA.

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