Supplementary Discussion

Extrasynaptic $GABA_A$ receptors are morphologically distinct from synaptic $GABA_A$ receptors as, in the main, they contain the δ subunit. This conveys both an increased sensitivity to low concentrations of GABA and reduced desensitization properties compared with the γ2 subunit containing $GABA_A$ receptors located post-synaptically (Belelli *et al.* 2009). This morphological distinction is important as it confers on the extrasynaptic $GABA_A$ receptors compared with the synaptic $GABA_A$ receptors a relative insensitivity to benzodiazepines (in line with the lack of effect of lorazepam on 1ms SICI (Ziemann *et al.* 1996)) but an increased sensitivity to neurosteroids (in line with the cyclical changes in GABA observed by MRS ((Harada *et al.* 2010; Epperson *et al.* 2005)). The presence of an extrasynaptic GABAergic "tone" in humans has been confirmed in both pyramidal cells and interneurons within temporal neocortical brain slice preparations from epilepsy patients (Scimemi *et al.* 2006).

Our data do not provide a clear explanation as to *how* increased activation of the extrasynaptic $GABA_A$ receptors confers greater inhibition measured by 1ms SICI but we can speculate on possible mechanisms. Tonic GABA currents reduce spontaneous firing rates in interneurons (Semyanov *et al.* 2003), via an increase in chloride ion (Cl^-) conductance and therefore, as the equilibrium potential of Cl^- is lower than the resting membrane potential, hyperpolarize the membrane. However, hyperpolarization of the membrane leads to a *decrease* in the duration of the relative refractory period, (Kiernan & Bostock, 2000), meaning cells should be more responsive to the test stimulus, demonstrated as *reduced*

inhibition. The GABA-induced hyperpolarization of the membrane therefore cannot directly explain the relationship described here, where higher GABA levels are associated with greater inhibition at 1ms ISI, presumably reflecting a longer relative refractory period. However, the relationship may be explained by a difference in intracellular chloride ion concentration ($[Cl^-]_i$) between the cell body and the axons. TMS primarily acts on axons (Di Lazzaro *et al.* 2004). $[Cl^-]_i$ is higher in neuronal axons than in the neuronal cell bodies, and critically, is found at a concentration high enough to generate a potential greater than the reversal potential of Cl^- (Khirug *et al.* 2008; Stell, 2011). This means that, at the axon, an influx of Cl^- will cause depolarization rather than hyperpolarization of the cell (Khirug *et al.* 2008; Rojas *et al.* 2011), which will lengthen the relative refractory period (Kiernan & Bostock, 2000), thus explaining the relationship demonstrated in our data.

Figure Legends

Supplementary Figure 1

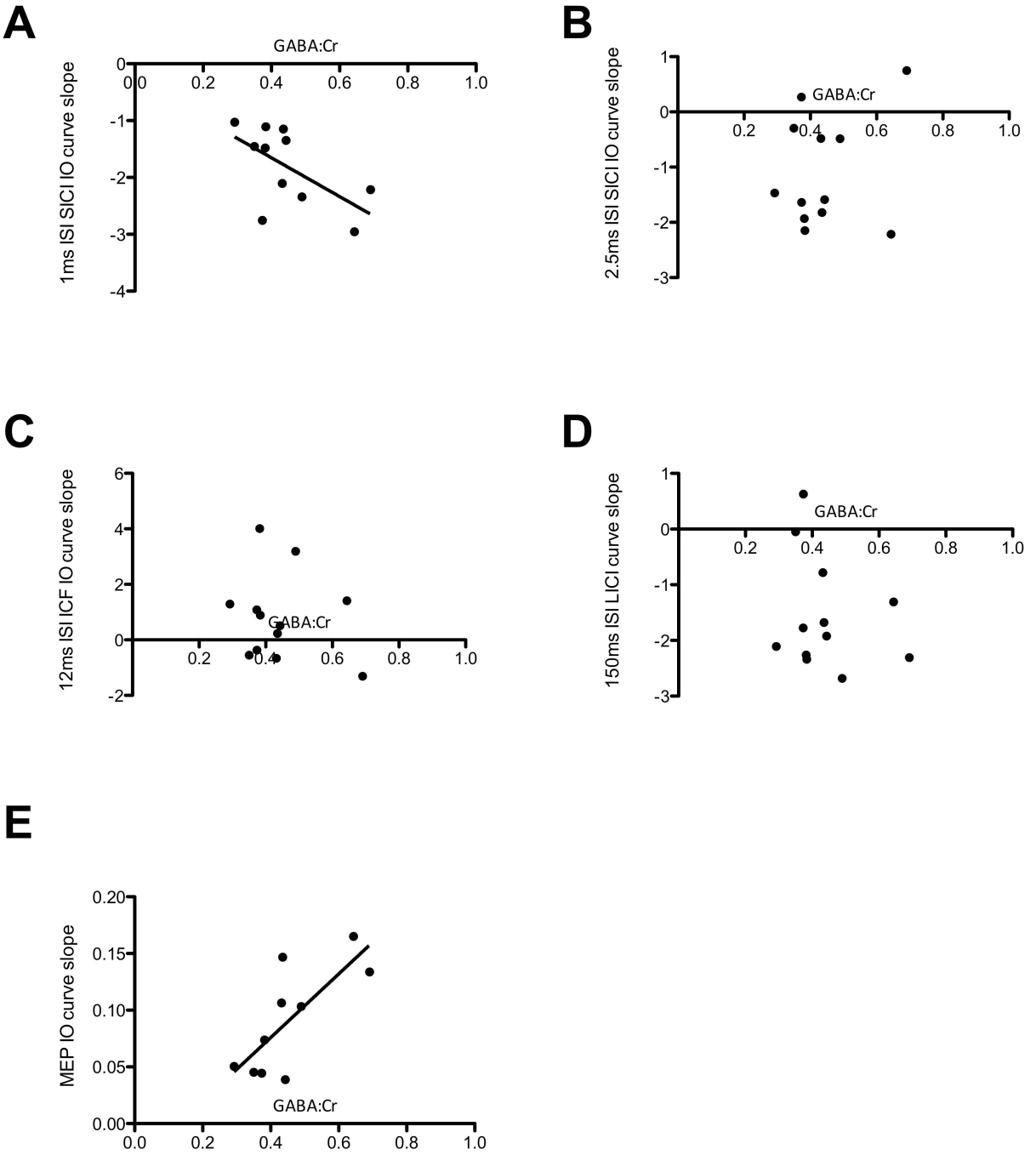
Summary figure of all correlations performed between MRS-GABA and TMS measures. A significant relationship was observed between MRS-GABA and the 1ms SICI measure (A) and between MRS-GABA and MEP IO curve (E), although this was in a counter-intuitive direction, with higher GABA levels being correlated with steeper IO curves. The remaining relationships were non-significant.

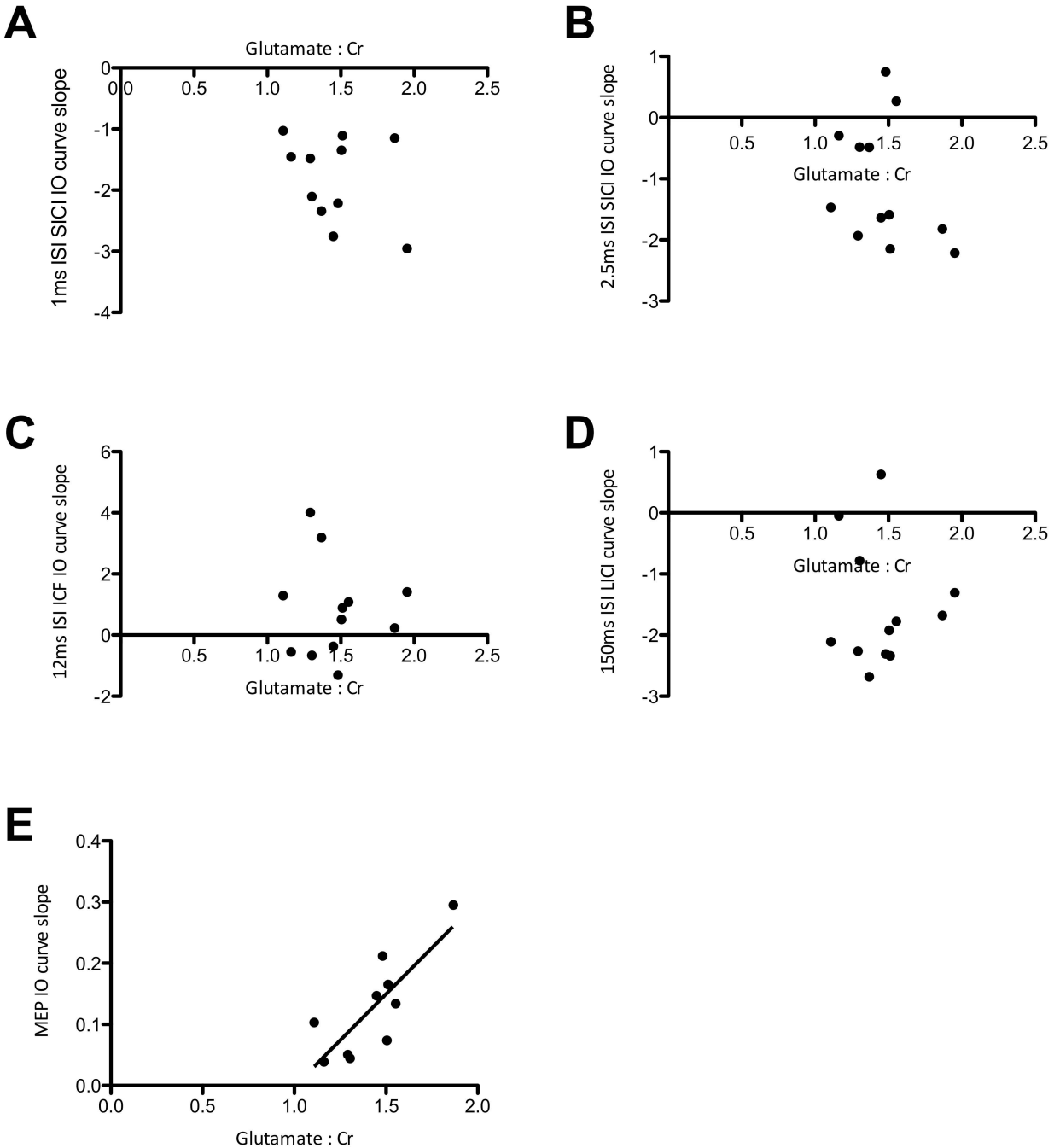
Supplementary Figure 2

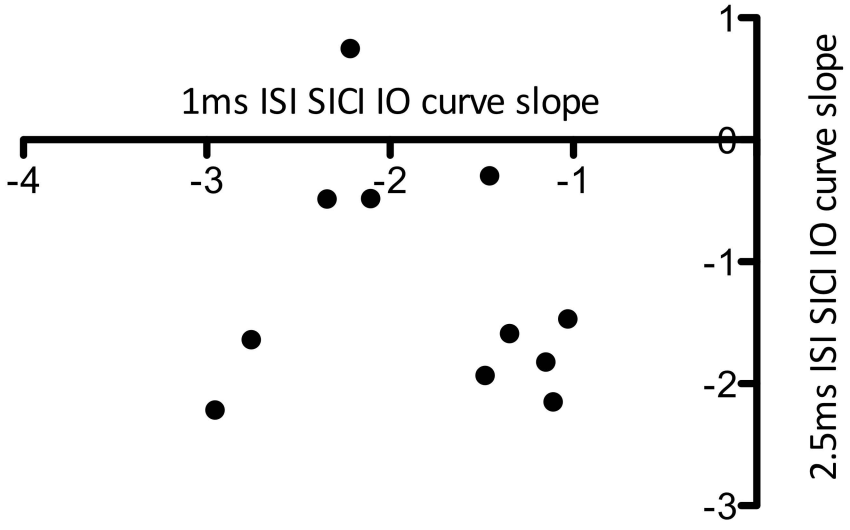
Summary figure of all correlations performed between MRS-Glutamate and TMS measures. A significant relationship was observed between MRS-Glutamate and MEP IO curve (E). The remaining relationships were non-significant.

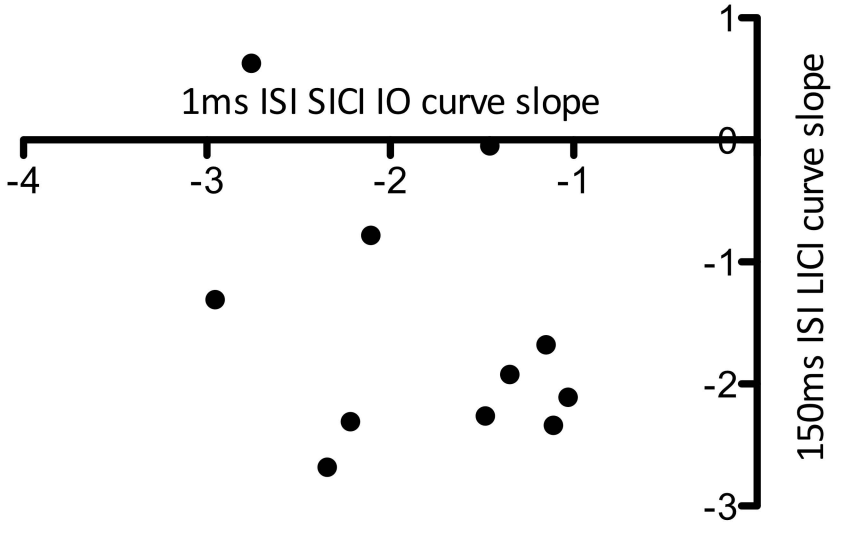
Supplementary Figure 3

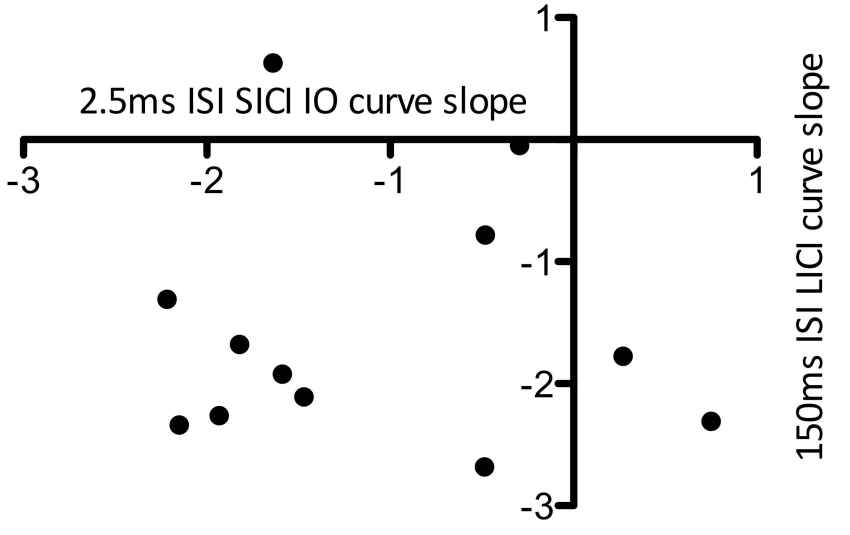
Summary figure of all correlations performed between the TMS inhibitory measures. All relationships were non-significant.


A
GABA:Cr
1ms ISI SICI IO curve slope
0
-1
-2
-3
-4
0.0
0.2
0.4
0.6
0.8
1.0
B
GABA:Cr
2.5ms ISI SICI IO curve slope
1
0
-1
-2
-3
0.2
0.4
0.6
0.8
1.0
C
GABA:Cr
12ms ISI ICF IO curve slope
6
4
2
0
-2
0.2
0.4
0.6
0.8
1.0
D
GABA:Cr
150ms ISI LICI curve slope
1
0
-1
-2
-3
0.2
0.4
0.6
0.8
1.0
E
GABA:Cr
MEP IO curve slope
0.20
0.15
0.10
0.05
0.00
0.0
0.2
0.4
0.6
0.8
1.0


A
Glutamate : Cr
1ms ISI SICI IO curve slope
0
-1
-2
-3
-4
0.0
0.5
1.0
1.5
2.0
2.5
B
2.5ms ISI SICI IO curve slope
1
0
-1
-2
-3
0.5
1.0
1.5
2.0
2.5
Glutamate : Cr
C
12ms ISI ICF IO curve slope
6
4
2
0
-2
0.5
1.0
1.5
2.0
2.5
Glutamate : Cr
D
150ms ISI LICI curve slope
1
0
-1
-2
-3
0.5
1.0
1.5
2.0
2.5
Glutamate : Cr
E
MEP IO curve slope
0.4
0.3
0.2
0.1
0.0
0.0
0.5
1.0
1.5
2.0
Glutamate : Cr


1ms ISI SICI IO curve slope
2.5ms ISI SICI IO curve slope
-4
-3
-2
-1
0
1
-1
-2
-3


1ms ISI SICI IO curve slope
150ms ISI LICI curve slope
-4
-3
-2
-1
0
1
-1
-2
-3


2.5ms ISI SICI IO curve slope
150ms ISI LICI curve slope
-3
-2
-1
1
-1
-2
-3