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## The contribution of GABA<sub>B</sub> circuits in the pro-epileptogenic action of nicotinic acetylcholine receptors in hippocampus

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### Abstract

**Background & Aims:** Nicotinic acetylcholine receptors (nAChRs) regulate epileptiform discharges generated by a number of different pharmacological manipulations, in the hippocampus. Functional nicotinic receptor-mediated responses are most prominently observed in hippocampal interneurons, however the pro-epileptogenic action of nAChRs appears to be independent of fast GABAergic transmission since it is resistant to the blockade of GABA<sub>A</sub> receptors. To identify possible contribution of the GABA<sub>B</sub> receptor in the nAChR-induced effect, a set of experiments was carried out in the presence of GABA<sub>B</sub> receptor antagonist CGP55845A.

**Material and Methods:** Hippocampal slices (400µm thick) were prepared from male Wistar rats (3-4 weeks). Following a 1hr equilibration in artificial cerebrospinal fluid, slices were transferred to a submerged-type recording chamber. Extracellular field recordings were made in the Stratum pyramidale of the CA3 regions. Bath application of the convulsant compounds 4-aminopyridine (4AP) or bicuculline resulted in the development of spontaneous epileptiform bursting.

**Results:** Slices pre-incubated with CGP55845A (GABA<sub>B</sub> receptor antagonist) were not able to exhibit burst frequency potentiation of 4AP-induced epileptiform activity upon application of the selective nAChR agonist dimethylphenylpiperazinium iodide (DMPP; -8.3 ± 7%, n=11). Similarly, in the presence of CGP55845A, slices exhibited negligible burst frequency potentiation of bicuculline-induced epileptiform activity upon DMPP application (27.6 ± 18%, n=9), in comparison to those observed in the absence of CGP55845A (248 ± 76 %, n=14). The suppression of epileptiform activity by the GABA<sub>B</sub> receptor agonist baclofen, was partially recovered by subsequent co-application of DMPP (n=10).

**Conclusion:** These data suggests that nAChRs may regulate the excitability of hippocampal networks at least in part through GABA<sub>B</sub> receptor-mediated mechanisms.

**Keywords:** Acetylcholine, Nicotinic, Epilepsy, GABA<sub>B</sub>, Hippocampus

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### Introduction

Much of the action of acetylcholine within the hippocampus is thought to be mediated through action on the GABAergic system (1). The hippocampus receives a dense cholinergic innervation from the medial septal nucleus which contacts both interneurons and principal cells (2, 3). The anatomical results, taken together with the electrophysiological data have shown that functional nicotinic receptors are present on

hippocampal interneurons (4-8). Located at pre, post and extra-synaptic sites of interneurons, nAChRs are ideally placed to modulate inhibitory circuits in the hippocampus. ACh increases the activity of interneurons via nicotinic receptors located in the somatodendritic membrane of interneurons (4, 6, 7) and increases GABA release through presynaptic nicotinic receptors located on axon terminals (9).

GABA<sub>B</sub> receptor-mediated mechanisms are involved in the generation of focal seizures and in epileptogenesis (10-12). Presynaptic GABA<sub>B</sub> receptors suppress neurotransmitter release (13). Depending on whether this action is exerted in GABAergic or glutamatergic neurons, there may be anticonvulsant or proconvulsant actions. Baclofen is a GABA<sub>B</sub> receptor agonist which has been shown to activate both pre- and post-synaptic GABA<sub>B</sub> receptors (14, 15) and to inhibit many forms of synaptic transmission (16) including glutamatergic (17), GABAergic (18) and cholinergic transmission (19). Baclofen may possess a proconvulsant effect as documented in clinical practice (20, 21) and in certain models of epileptiform discharge (22-24). It has also been proposed that the proconvulsant effect of baclofen is caused by a presynaptic, GABA<sub>B</sub>-mediated inhibition of GABA release from inhibitory interneurons leading to disinhibition (22, 23).

Previous results from our laboratory have shown pharmacological activation of nicotinic acetylcholine receptors (nAChRs) to produce a sustained pro-epileptogenic effect within the hippocampal slice (Roshan-milani et al 2003). This was observed across a range of different forms of pharmacologically-induced epileptiform activity, including 4-aminopyridine (4AP), bicuculline and low magnesium models, suggesting that nAChRs may have a general action in exacerbating epilepsy-like discharges. The mechanisms underlying the pro-epileptogenic action of nAChRs are complex and not simply due to the activation of nAChRs, and a number of factors may contribute to their pro-epileptogenic actions. An important locus of action is likely to be GABAergic circuits. nAChRs can have a potent impact upon the activity of interneurone populations which themselves are important regulators of network excitability. Thus, it is likely that the pro-epileptogenic action of nAChR activation is mediated in part through GABAergic circuits. However, such an action of nAChRs appears to be independent of fast GABAergic transmission since the pro-epileptogenic actions of nAChR activation is resistant to the blockade of GABA<sub>A</sub> receptors (Roshan-Milani et al, 2003). Thus, nAChR modulation of

GABAergic events is unlikely to be important in the pro-epileptogenic action of nicotinic receptor activation, although it is possible that an interaction between nAChRs and GABA<sub>B</sub> receptor activation may play a role. The aim of the present study, therefore, was to readdress the possible contribution of GABA<sub>B</sub> circuits in nAChRs-induced burst frequency potentiation and to further investigate the effect of the nAChR agonist on GABA release from hippocampal neurones with a view to uncover possible mechanisms responsible for the pro-epileptogenic action of nAChR.

## Materials and Methods

### *Slice Preparation:*

Experiments were performed on transverse hippocampal slices obtained from male Wistar rats (3-5 weeks old). Following cervical dislocation, animals were decapitated in accordance with U.K. Home Office Guidelines. The brain was removed rapidly and immersed in a beaker containing chilled artificial cerebrospinal fluid (ACSF). Transverse horizontal slices were prepared as described previously (25) by hemisecting whole brain minus the cerebellum and cutting 400  $\mu$ m thick transverse slices using a vibrating microtome (Leica VT1000, Milton Keynes, U.K.). The hippocampal formation was cut free from the surrounding brain areas using a scalpel blade and the resultant slices were placed on a lens tissue at the interface of a warmed (32-34 °C) artificial cerebrospinal fluid (perfusion rate 1-2 ml.min<sup>-1</sup>) and an oxygen-enriched (95% O<sub>2</sub>, 5% CO<sub>2</sub>), humidified atmosphere. The standard perfusion medium (ACSF) comprised (mM): NaCl, 124; KCl, 3; NaHCO<sub>3</sub>, 26; NaH<sub>2</sub>PO<sub>4</sub>, 1.25; CaCl<sub>2</sub>, 2; MgSO<sub>4</sub>, 1; D-glucose, 10; and was bubbled with 95% O<sub>2</sub>, 5% CO<sub>2</sub>.

### *Extracellular field recordings:*

Extracellular recording electrodes were pulled from standard wall borosilicate tubing using a Brown and Flaming type horizontal electrode puller (Sutter Inst, U.S.A.). Recording electrodes were filled with ACSF and exhibited a d.c. resistance of 1-5 M $\Omega$ . Slices were allowed to equilibrate in the recording chamber for at least one hour before recording commenced. In order to

measure synchronous neuronal discharges, electrodes were placed close to stratum pyramidale within area CA3 (usually CA3c) of the hippocampal slice. All recordings were performed under current clamp conditions and signals amplified using an Axoclamp 2B amplifier (Axon Instruments, U.S.A.) operated in bridge mode. Signals were further amplified and conditioned using a Brownlee Model 440 signal processor (Brownlee Inst., San Jose, CA, U.S.A) and data captured directly onto DAT tape (DTR-1404; Biologic Scientific Instruments, Claix, France) and/or onto a PC hard disk using pClamp8.0 software (Axon Instruments, CA., U.S.A.).

#### **Data analysis:**

Analysis was carried out off-line using pClamp8, Origin6 (Microcal, MA. U.S.A.), Mini Analysis version 5.6.28 (Synapsoft Inc. GA, USA) and Igor Pro3.1 (Wavemetrics, OR., U.S.A) software packages. Data are presented as means (standard error of the means (S.E.M.) and statistical significance determined using paired Students *t*-tests or ANOVA performed on raw data with  $P < 0.05$  being taken as indicating statistical significance. *n* values refer to the number of times a particular experiment was repeated.

#### **Drugs:**

All pharmacological agents were purchased from Sigma (Poole, U.K.) except bicuculline and baclofen which was purchased from Research Biochemicals International (Natick, MA, USA.). [1-(*S*)-3, 4-dichlorophenyl]ethyl]amino-2-(*S*)-hydroxypropyl-p-benzyl-phosphoic acid (CGP 55845A) was gift from Dr KumleshDev, Novartis Pharmaceuticals, Basel, Switzerland. In all experiments, drugs were applied by addition to the perfusion medium.

## **Results**

### ***Effect of nAChR activation on 4AP-induced epileptiform activity in the presence of GABA<sub>B</sub> receptor antagonist:***

Our group have already shown that, activation of nAChRs potentiates pre-established epileptiform bursting activity in the hippocampus (25). We had shown

that co-application of the selective nAChR agonist DMPP (30  $\mu$ M) on ongoing epileptiform activity generated by a number of different pharmacological manipulations, including 4AP, results in an increase in burst frequency (Fig. 1;  $n=31/37$ ).

In order to investigate the possible involvement of GABA<sub>B</sub> receptors in nAChRs-induced effect, a series of experiments were carried out in which the slices were pre-incubated with GABA<sub>B</sub> receptor selective antagonist CGP55845A prior to application of DMPP. Once stable 20  $\mu$ M 4AP-induced bursting was established, 1  $\mu$ M CGP55845A was co-applied in order to block pharmacologically any involvement of GABA<sub>B</sub> receptor mediated responses during epileptiform bursting. Such application of CGP55845A did not significantly change the frequency of 4AP-induced bursting activity (mean frequency = 97.8 (4% of control 4AP frequency;  $P > 0.05$ ;  $n=11$ ; Fig.2 A-B). After the stabilisation of the 4AP-induced spontaneous epileptiform activity in the presence of CGP55845A, 30  $\mu$ M DMPP was co-applied to the convulsant containing solution to test the effect of nAChR activation on ongoing 4AP-induced epileptiform activity when GABA<sub>B</sub> receptors are blocked. Whereas slices in the absence of CGP55845A would exhibit robust burst frequency potentiation (Fig.1), slices pre-incubated with CGP55845A (1  $\mu$ M) failed to exhibit burst frequency potentiation upon DMPP application in 11 of 11 slices tested. In the presence of CGP55845A, application of DMPP resulted in no significant enhancement of burst frequency compared to pre-DMPP levels (91.7 (7% of pre-DMPP control;  $P > 0.05$ ;  $n=11$ ; Fig.2 A-B). Overall, these data suggest that GABA<sub>B</sub> receptors may play a role in nAChRs-induced frequency potentiation of 4AP-induced bursting.

### ***Effect of nAChR activation on BIC-induced epileptiform activity in the presence of GABA<sub>B</sub> receptor antagonist:***

To assess the effect of GABA<sub>B</sub> receptor antagonism on ongoing BIC-induced epileptiform discharges and their modulation by nAChRs-induced effect, CGP55845A was bath applied to slices displayed stable bicuculline-induced epileptiform burst activity.

Subsequent co-application of CGP55845A (1-3  $\mu$ M, n=9) to hippocampal slices generating interictal discharges under control conditions (BIC-containing medium) produced no significant change in burst frequency (mean maximal frequency = 106.8 (5% of control BIC frequency,  $P>0.05$ , Fig.3 A-B).

Once stable epileptiform activity was established, 30  $\mu$ M DMPP was co-applied in order to investigate the effect of nAChRs activation on ongoing BIC-induced epileptiform discharges in the presence of CGP55845A. As detailed previously (25), BIC-induced epileptiform activity showed a profound frequency potentiation by subsequent co-application of the selective nAChR agonist DMPP (248 (76% of baseline; n=14). In contrast, slices pre-incubated with the GABA<sub>B</sub> receptor antagonist CGP55845A (1-3  $\mu$ M) exhibited negligible burst frequency potentiation upon DMPP application (27.6  $\pm$ 17.76% of baseline;  $P>0.05$ ; n=9; Fig.3 A-C). Although under this condition, there was no change in the frequency of BIC-induced epileptiform bursting during DMPP application in the majority of slices tested (n=6 out of 9 experiments, Fig.3 A), a small increase was observed in the other 3 experiments (Fig.3 B). However, these changes did not achieve statistical significance (One-way ANOVA,  $P=0.18$ , Fig.3 C).

#### ***Effect of GABA<sub>B</sub> receptor activation on 4AP-induced epileptiform activity:***

Application of the GABA<sub>B</sub> receptor agonist baclofen (0.1-30 $\mu$ M) resulted in a concentration dependent reduction and eventual complete blockade of 4AP-induced interictal activity (n=15, Fig.4 and 5). The effects induced by increasing concentration of baclofen were analysed in 8 slices. With 0.1-10 $\mu$ M baclofen, a decrease in the rate of occurrence of interictal discharges occurred in 5 of 8 slices, whereas abolishment was seen in the remaining 3 experiments. In slices in which interictal activity was not fully abolished by 10 $\mu$ M baclofen, further increasing the concentration to 30 $\mu$ M caused a complete abolishment (n=5 of 5). Complete blockade of interictal discharges were also induced by application of a single concentration of baclofen (30  $\mu$ M, n=7, Fig.5).

The suppressant actions of baclofen upon epileptiform bursting were fully reversible upon washout (n=3, Fig.4A) or co-application of GABA<sub>B</sub> receptor antagonist CGP55845A (1 $\mu$ M, n=3, Fig.4B). We also analysed the concentration-response relationship of the changes induced by baclofen with respect to the frequency of 4AP-induced epileptiform activity (Fig.4 C). Data obtained in 8 slices in which increasing doses of baclofen(0.1-30 $\mu$ M) were sequentially applied indicated an $IC_{50}$  = 4.7  $\mu$ M, comparable with  $IC_{50}$  value of other studies (26, 27). Finally, in a minority of slices tested 30  $\mu$ M baclofen application resulted in abolishment of interictal activity and the appearance of spontaneous ictal activity which was absent in control periods (n = 2 of 17, Fig.6 B).

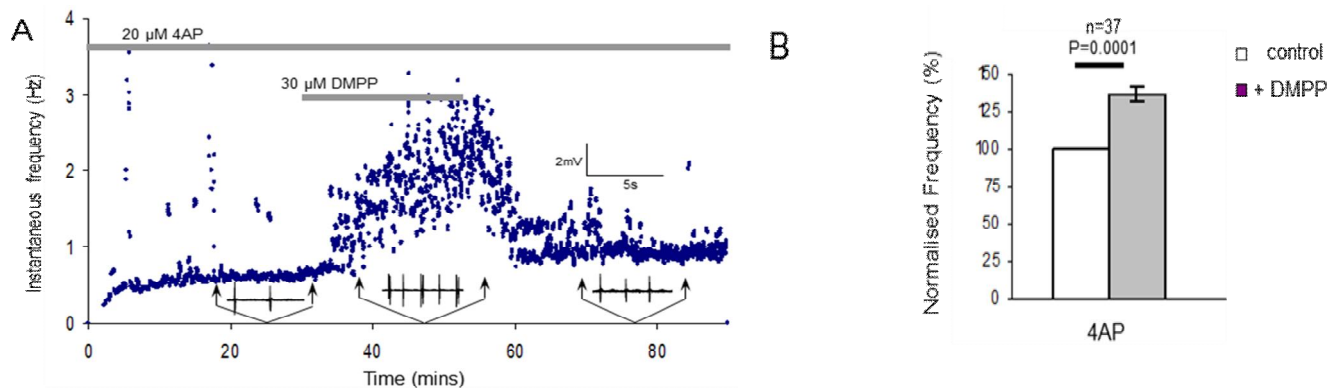
#### ***Effect of nAChR activation on baclofen-induced suppression of epileptiform activity:***

In order to assess whether nAChR activation could modulate baclofen-induced suppression of epileptiform activity, a series of experiments were carried out in which DMPP was co-applied following a stable periods of baclofen application. When the nAChR agonist DMPP (30  $\mu$ M) was applied to slices treated with 4AP and baclofen, interictal activity reappeared upon DMPP application (n=10, Fig.5 A-C). Although baclofen-induced suppression of 4AP-induced epileptiform activity was reversed upon application of DMPP, the degree of reversal varied between experiments. In 7 out of 10 experiments, application of 30  $\mu$ M DMPP partially recovered the epileptiform bursting activity but the frequency recovery did not achieve pre-baclofen levels (39.3 (5% of control, Fig.5 A-B). Further reverse of baclofen-induced effects achieved following co-application of CGP55845A to medium containing 4AP, baclofen and DMPP (Fig.5 A-B).In the remaining 3 slices application of DMPP resulted in a complete reverse of baclofen effects to pre-baclofen frequency levels (95 (4.5% of control, Fig.5 C).

As it is mentioned above, baclofen application (30  $\mu$ M) elicited ictal-like discharges (which were absent in control) in 2 out of 17 experiments. In all these cases, subsequent application of 30  $\mu$ M DMPP resulted in the

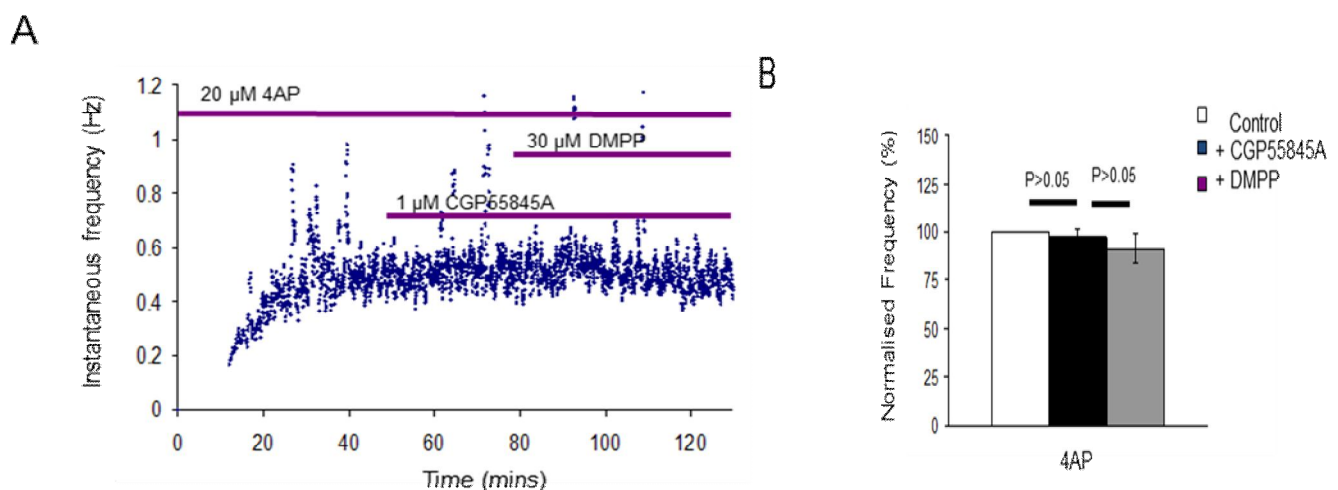
subsequent reappearance of interictal activity whereas the rate of occurrence of the ictal-like events decreased (from 0.17 (0.02 Hz during 30  $\mu$ M baclofen to 0.08 (0.02 Hz after adding DMPP, n=2; Fig.6). DMPP also reduced

the duration of the ictal-like discharges that occurred less frequently than with baclofen only (from 634.8  $\pm$ 26ms during 30  $\mu$ M baclofen to 321 (19 ms after adding DMPP, n=2).



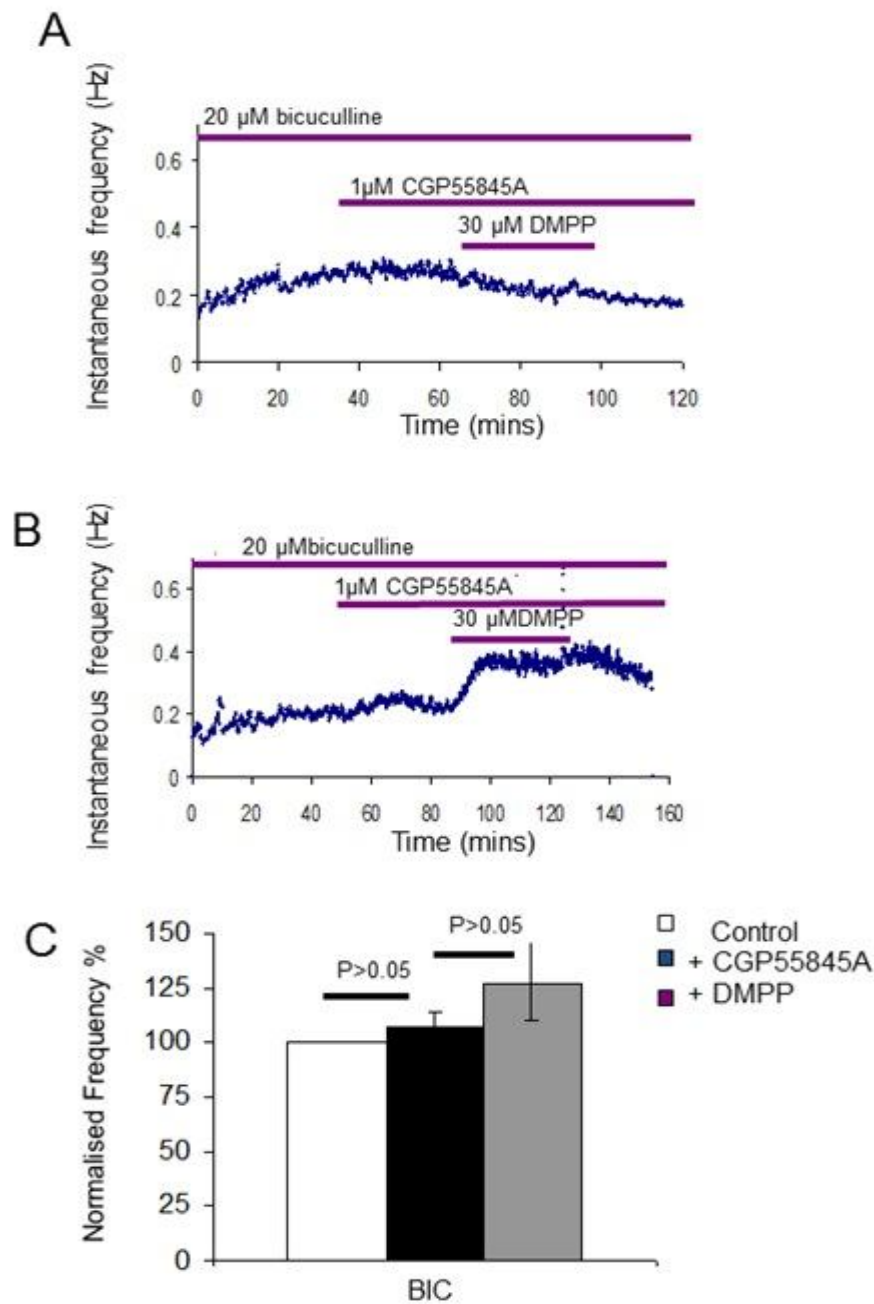
**Fig. 1:** Effect of nAChR agonists on 4AP-induced epileptiform bursting in area CA3 of the hippocampus.

A. Scatter plot showing instantaneous burst frequency in response to continuous application of 20 $\mu$ M4AP throughout a representative experiment. Co-application of the selective nAChR agonist DMPP (30  $\mu$ M) for the period indicated by the horizontal bar resulted in an increase in burst frequency. Inset voltage traces show rhythmic bursting in the extracellular field potentials before, during and after DMPP application at the times indicated by arrows. Scales 2mV, 5s. B. Summary of the effects of application of DMPP on 4AP-induced burst frequency (n=37). Horizontal bars indicate P values between respective columns as determined using Wilcoxon-matched pair test.



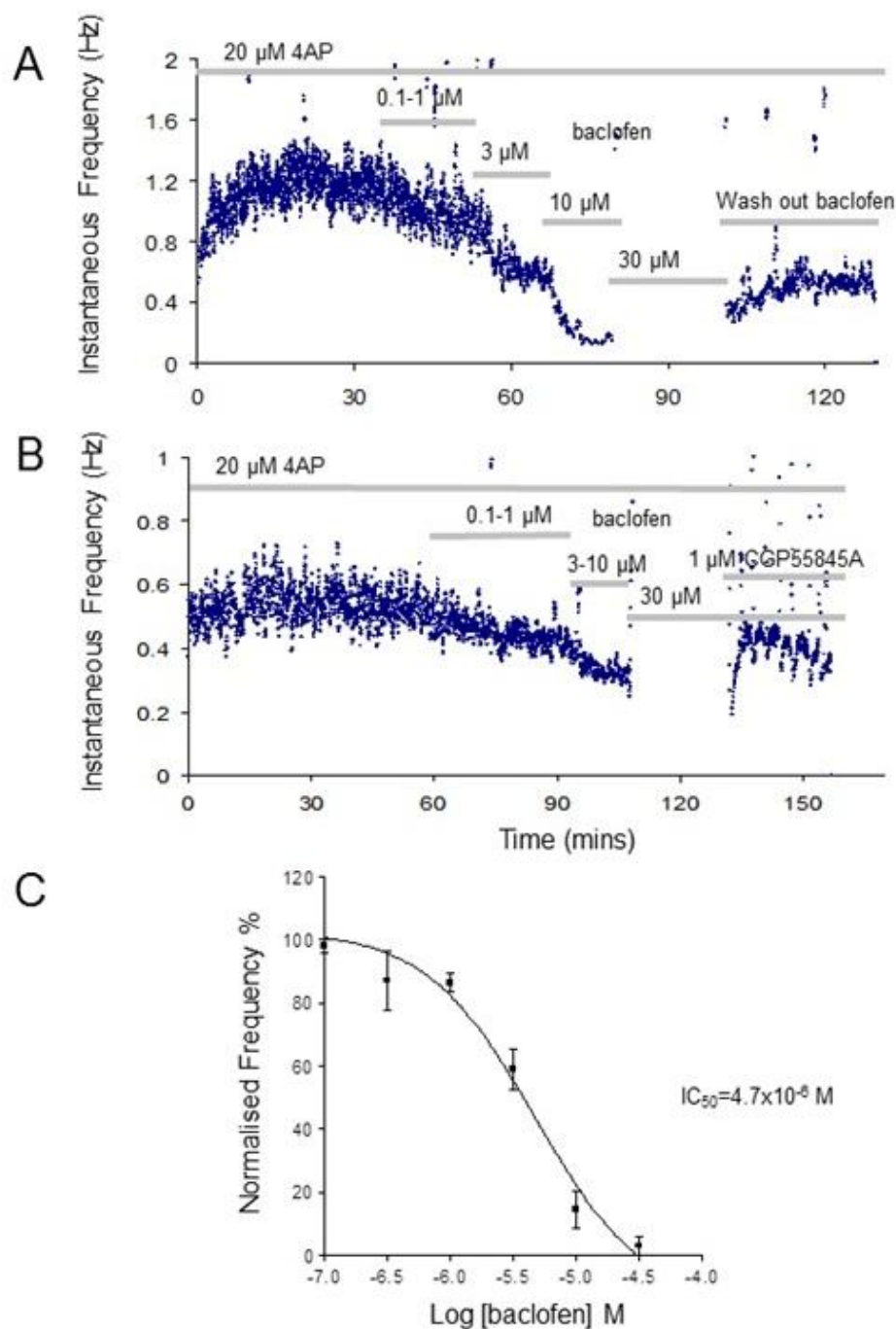
**Fig. 2:** Effect of nAChR activation on epileptiform activity in the presence of GABA<sub>B</sub> receptor antagonist.

A. Scatter plot showing the effect of DMPP on 4AP-induced burst frequency in the presence of GABA<sub>B</sub> receptor antagonist, CGP55845A. Co-application of 1  $\mu$ M CGP55845A did not produce any significant change in 4AP-induced epileptiform activity. Subsequent co-application of DMPP again produced no significant change in 4AP-induced burst frequency in the presence of CGP55845A. B. Summary of the effects of application of DMPP on 4AP-induced burst frequency in the presence of CGP55845A (n=11). This GABA<sub>B</sub> receptor antagonist prevents the ability of DMPP to potentiate 4AP-induced epileptiform burst frequency. Horizontal bars indicate P values between respective columns as determined using ANOVA.



**Fig. 3:** Effect of nAChR activation on BIC-induced epileptiform activity in the presence of GABA<sub>B</sub> receptor antagonist.

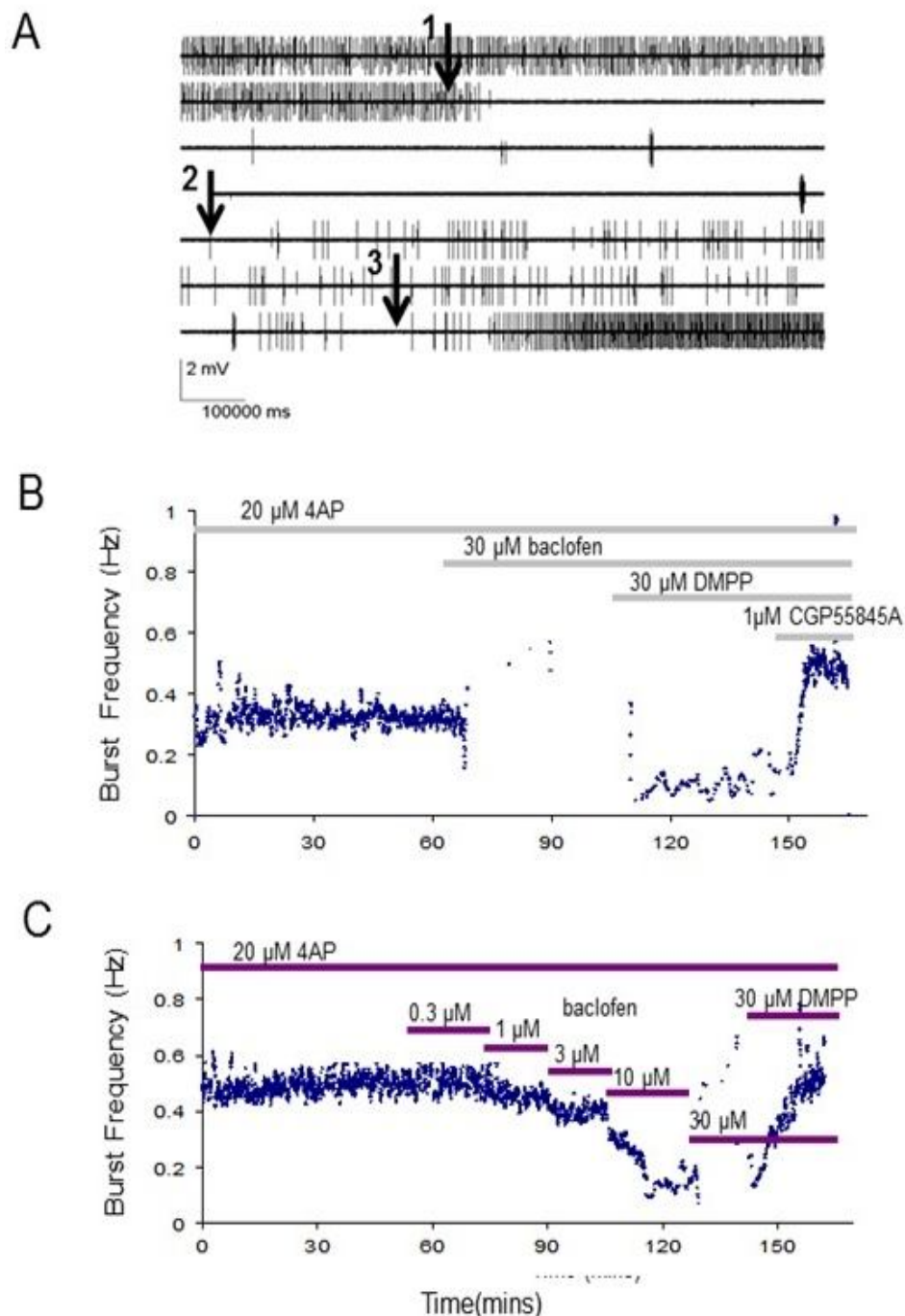
A. Scatter plot showing the effect of DMPP on BIC-induced burst frequency in the presence of GABA<sub>B</sub> receptor antagonist, CGP55845A. Co-application of 1  $\mu$ M CGP55845A did not produce any significant change in BIC-induced epileptiform burst frequency. In the majority of the slices, subsequent co-application of DMPP again did not produce any change in BIC-induced epileptiform activity (n=6 of 9). B. In a minority of slices (n=3 of 9) subsequent co-application of DMPP produced a modest burst frequency potentiation of BIC-induced epileptiform activity but which overall did not reach statistical significance (P=0.18, One-Way ANOVA). C. Summary of the effects of application of DMPP on BIC-induced burst frequency in the presence of CGP55845A across all slices tested (n=9). Horizontal bars indicate P values between respective columns as determined using ANOVA.



**Fig. 4:** Effect of GABA<sub>B</sub> receptor agonist baclofen on 4AP-induced epileptiform activity in area CA3 of the hippocampus.

A. Scatter plot showing effect of baclofen on frequency of interictal-like events induced by 4AP in the rat hippocampal slice. Application of baclofen (0.1-30  $\mu$ M) resulted in a concentration-dependent decrease and eventual complete abolition of the 4AP-induced interictal-like activity. Shaded horizontal bars indicate the period over which an individual concentration was applied to the bath. B. Similar scatter plot in which the effect of 30  $\mu$ M baclofen is reversed upon co-application of the GABA<sub>B</sub> receptor antagonist, CGP55845A (1  $\mu$ M). C. Concentration-response curve for the decrease in the frequency of 4AP-induced interictal activity, induced by different concentrations of baclofen in 8 experiments. This dose-response curve reveals an  $IC_{50}$  of 4.7  $\mu$ M.

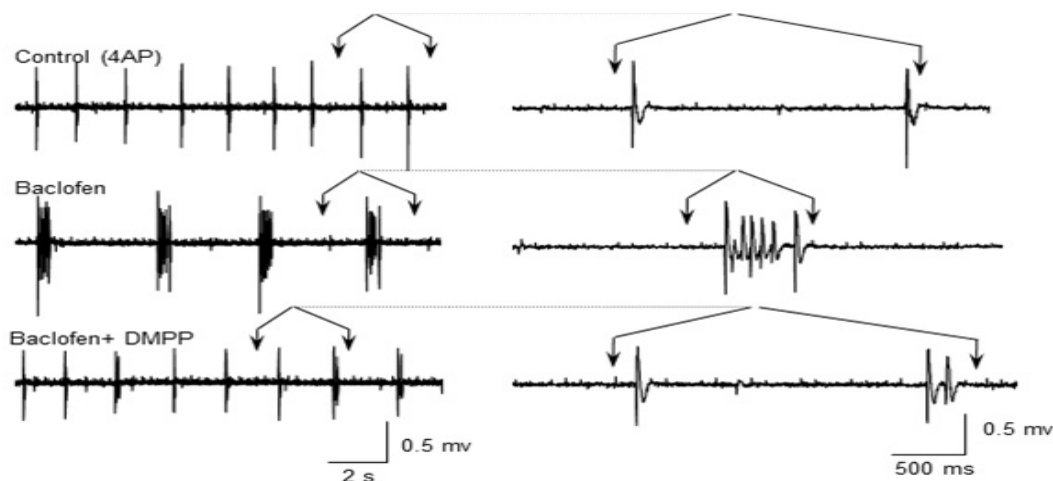




**Fig. 5:** Reverse of baclofen-induced effects on epileptiform activity by DMPP.

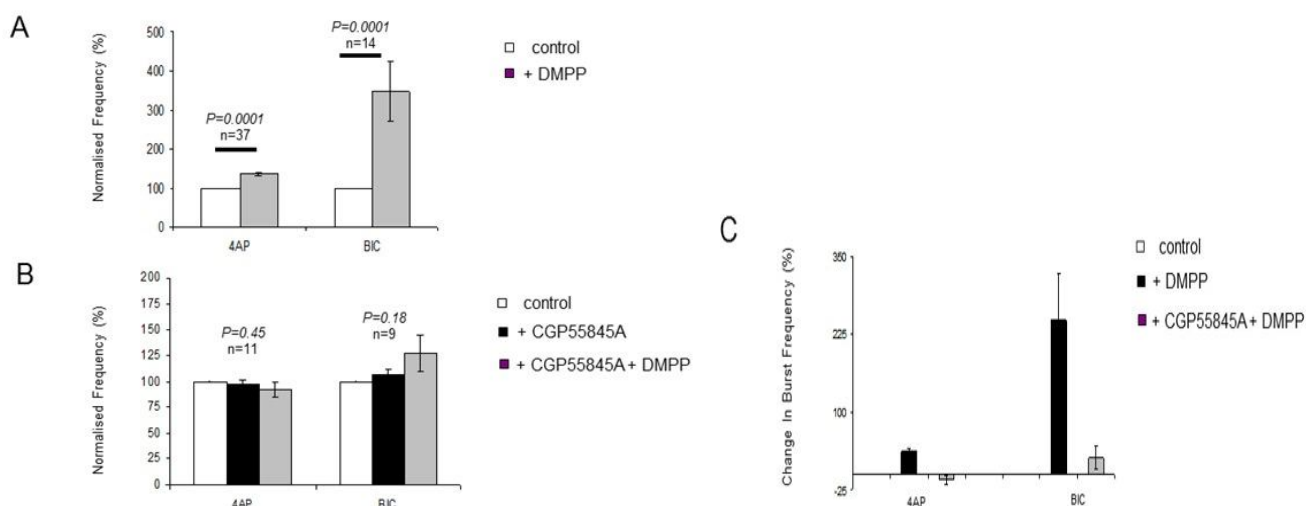
A. Raster plot showing a representative experiment in which interictal bursting activity induced by 4AP is completely abolished following application of baclofen (30  $\mu$ M, arrow 1 onwards). Subsequent co-application of DMPP (30  $\mu$ M, arrow 2) and CGP55845A (1  $\mu$ M, arrow 3) recovered and then potentiated interictal activity, respectively. B. Scatter plot presentation of same experiment showing application of a single concentration of baclofen (30  $\mu$ M) abolishes 4AP-induced interictal activity which is partially reversible upon co-application of DMPP (30  $\mu$ M, n=7 of 10). Further reverse of baclofen-induced effects achieved following co-application of CGP55845A to medium containing 4AP, baclofen and DMPP. C. In 3 out of 10 experiments application of DMPP resulted in a complete reverse of baclofen effects to pre-baclofen frequency levels.





**Fig. 6:** Effect of DMPP on baclofen-induced effect on epileptiform activity.

In a minority of experiments baclofen abolished interictal events, while promoting the occurrence of more prolonged ictal-like after discharges (n=2 of 17). In these cases, DMPP application resulted in the reappearance of interictal activity and a shortening in the duration and the rate of occurrence of the ictal events. Right column points to expanded sections of each trace illustrating individual field potential events.



**Fig. 7:** Comparison of DMPP-induced burst frequency potentiation in 4AP and BIC models of epileptiform activity in the absence and presence of GABA<sub>B</sub> receptor antagonist.

A. Histogram summarising 30  $\mu$ M DMPP-induced burst frequency potentiation in 4AP and BIC models normalised to control (pre-DMPP) frequency. In the absence of GABA<sub>B</sub> receptor antagonist, activation of nAChRs by DMPP results in a significant increase in burst frequency in both models (Wilcoxon-matched pair test,  $P < 0.01$ ). B. Quantitative summary of the effect of DMPP on 4AP and BIC-induced epileptiform activity in the presence of GABA<sub>B</sub> receptor antagonist, CGP55845A (1-3  $\mu$ M). Application of CGP55845A blocked the ability of DMPP to potentiate 4AP-induced bursting (n=11) and also substantially decreased DMPP-induced maximal burst frequency potentiation in BIC model (n=9). One-Way ANOVA indicated no significant change in burst frequency upon application of DMPP in the presence of CGP 55845A in 4AP ( $P = 0.45$ ) and BIC models ( $P = 0.15$ ). C. Histogram summarising DMPP-induced change in burst frequency in the absence and presence of CGP55845A. Note the maximal burst frequency potentiation in BIC model in the absence of CGP55845A is 200 fold higher in comparison to those in the presence of this antagonist.

## Discussion

The main finding of this study is the demonstration that preincubation of slices with a GABA<sub>B</sub> receptor antagonist can prevent the ability of the nAChR agonist DMPP to induce epileptiform burst frequency potentiation. This is consistent with the proposal that nAChRs might affect epileptiform activity in part through modulating GABAergic circuits. GABAergic interneurons in the hippocampus are known to exhibit functional nAChR-mediated responses (4, 5, 7, 8). Strong neurochemical evidence indicates that presynaptic nAChRs are involved in the enhanced release of a number of transmitters, including GABA (9, 28, 29). Given these evidences, it is likely that the pro-epileptogenic action of nAChRs is mediated in part through GABAergic circuits. However, such an action of nAChRs appears to be independent of fast GABAergic transmission. An important finding in this respect was that nAChR activation also resulted in a facilitation of epileptiform burst discharge in the bicuculline model (Roshan-Milani et al., 2003) in which GABA<sub>A</sub> receptors are blocked by high concentrations of the specific GABA<sub>A</sub> receptor antagonist. Thus it seems that such an action of nAChRs appears to be independent of fast GABAergic transmission. Indeed, this agrees well with a previous report showing the ability of nicotine to potentiate paroxysmal depolarising shifts following GABA withdrawal syndrome (30). However, an action on GABA<sub>B</sub> receptor mediated synaptic inputs cannot be excluded since interaction between nAChRs and GABA<sub>B</sub> receptors may impact on this cellular process. Chronic nicotine modulates the GABA<sub>B</sub> receptors expression of rat prefrontal cortex(31) and GABA<sub>B</sub>-induced calcium signalling in cultured enteric neuron(32), implying a probable contribution of these receptors in nAChRs pharmacological effects.

GABA<sub>B</sub> receptor-mediated mechanisms are involved in the generation of focal seizures, in epileptogenesis (10-12) and also in facilitating epileptiform activity (24, 33). Baclofen, a GABA<sub>B</sub> receptor agonist, inhibits many form of synaptic transmission, however it may have a surprising proconvulsant effect caused by a presynaptic,

GABA<sub>B</sub>-mediated inhibition of GABA release from inhibitory interneurons (22, 23). Such an action has also been documented in 4AP-induced epileptiform activity; leading to hypothesize that GABA<sub>B</sub> receptor antagonist could exert anticonvulsant actions. Previous studies have shown that the GABA<sub>B</sub> receptor antagonist CGP35348 has anticonvulsant actions in rodent models of absence seizures (34, 35). However, some pro-convulsive effects of CGP35348 and CGP55845A have also been reported (33, 36), suggesting that the blockade of presynaptic GABA<sub>B</sub> receptor leads to an increase in GABA release and subsequent larger [K<sup>+</sup>] elevations (33). However, in our experiments, application of CGP55845A (1 μM) produced a very modest burst frequency potentiation in the BIC model and also did not change frequency of epileptiform activity in 4AP model. CGP 55845A at this concentration is known to completely block both presynaptic and postsynaptic GABA<sub>B</sub> receptors(37). However, it has been reported that GABA<sub>B</sub> receptors exist as subtypes having distinct neuronal locations, functions and pharmacological properties (For review see 38, 39). Indeed, different subtypes of GABA<sub>B</sub> receptors have been found at pre- and postsynaptic sites within the rat dorsolateral septal nucleus (40). If this GABA<sub>B</sub> receptor distinct pharmacology, distribution and strain-specific differences also extend to other CNS structures, such differences may therefore account for the findings described here.

Application of the GABA<sub>B</sub> receptor agonist baclofen decreased and eventually blocked the 4AP-induced interictal activity in all experiments. In our study most baclofen effects were antagonized by CGP55845A, thus indicating that they were mainly caused by the activation of GABA<sub>B</sub> receptors. In particular, we propose that baclofen abolishes 4AP-induced interictal activity by decreasing the release of transmitter from excitatory and inhibitory terminals. This may be caused by activation of presynaptic GABA<sub>B</sub> receptors inhibiting both GABA and excitatory transmitter release (17, 18) and by a postsynaptic GABA<sub>B</sub>-mediated hyperpolarization that decreases excitability of principal cells (15, 41) and

interneurons (42, 43). Reappearance of interictal activity by DMPP following application to medium containing 4AP and baclofen, could be as a result of effect of nAChR activation in enhancing neurotransmitter release from presynaptic terminals (28, 44, 45). However, this effect of DMPP cannot completely overcome the inhibition of transmitter release exerted by baclofen since the effect of baclofen was only partially reversible upon application of DMPP in most experiments. Subsequent application of CGP55845A blocked baclofen-induced inhibition of neurotransmitter release and produced additional enhancement of neurotransmitter release.

As detailed in previous report (Roshan-Milani et al., 2003), the frequency of 4AP and BIC-induced epileptiform activity were potentiated by subsequent co-application of the selective nAChR agonist, DMPP. In contrast, according to present data, slices pre-incubated with the GABA<sub>B</sub> receptor antagonist CGP55845A (1 μM) were not able to exhibit burst frequency potentiation upon DMPP application. Similarly, in the presence of 1 μM CGP55845A, slices exhibited negligible burst frequency potentiation of bicuculline-induced (20 μM) epileptiform activity upon DMPP application (27.6 (18%), in comparison to those observed in the absence of CGP55845A (248 (76%). A comparison of the effects of DMPP upon these two models of epileptiform bursting in the absence and presence of GABA<sub>B</sub> receptor antagonist is summarised in histogram format in figure 8. As illustrated in figure 8 C, the maximum burst frequency potentiation to DMPP application in 4AP and BIC models in the absence of CGP55845A is 45% and 220% higher than those in the presence of this antagonist, respectively. These data suggest that GABA<sub>B</sub> receptor activation is an important element in the nAChR-induced burst frequency potentiation and nAChRs may regulate the excitability of hippocampal networks through GABA<sub>B</sub> receptor-mediated mechanisms.

Overall the data presented in this study are robust and reproducible. However, the data at the same time appears

paradoxical and defy any simple mechanistic explanation. One possible explanation for this result is that increased nAChR function leads to an increase in GABA release from GABAergic interneurons. This leads to a subsequent recruitment of GABA<sub>B</sub> receptors, which in turn can modulate neuronal excitability via some mechanisms including: 1) Activation of postsynaptic GABA<sub>B</sub> receptors which can induce a membrane hyperpolarisation of inhibitory interneurons leading to disinhibition of pyramidal cells and increase in excitability. An activity-dependent change in CA3 area network excitability occurs during GABA<sub>B</sub> receptor activation (24). 2) Activation of presynaptic GABA<sub>B</sub> receptors which are involved in proepileptogenic action of nAChRs. Activation of presynaptic GABA<sub>B</sub> receptors induces presynaptic inhibition of GABA release on GABAergic terminals, which increase excitability.

In summary, the cholinergic activation of GABAergic circuits may recruit GABA<sub>B</sub> receptor-mediated modulation of hippocampal network states. Whilst we have found robust evidence that GABA<sub>B</sub> receptor activation is an important element in the nAChR-mediated burst frequency potentiation effect, the precise mechanistic detail is unclear and requires further investigation.

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### Conflicts of Interest:

The authors declare no conflict of interest.

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