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Medicinal plants: new uses & new mechanisms

Medicinal Plants:
From crop to cure
29th March 2011

Recent positive projects in MPRG with various plant extracts

Dementias

- *GINKGO (Ginko biloba)*
- SAGE (*Salvia officinalis)*
- LEMON BALM (*Melissa officinalis*)
- *GREEN TEA (Camellia sinensis)*

Depression

- ST JOHNS WORT **(***Hypericum perforatum)*

Alzheimers Disease

- Approx. 5% of people over 65 and 20% over 80 have dementia (700,000 UK)
- Many severely demented patients develop symptoms of agitation (severe restlessness, irritability and aggression)
- Antipsychotic drugs have been widely used to treat this

e.g. risperidone, donezepil, major tranquilizers

Side-effects:

Over-sedation

Social withdrawal/Reduced wellbeing

Falls

Further decrement in cognition

Possibly risk of stroke

URGENT NEED FOR ALTERNATIVE

Apart from cognitive therapy, increasing clinical evidence for efficacy and safety of **plant essential oils** for symptoms such as agitation

AROMATIC ESSENTIAL OIL THERAPY CONTROLLED CLINICAL TRIALS IN PEOPLE WITH DEMENTIA –up to 4 weeks

Mitchell et al 1993 **- 12 people, *Melissa* and lavender v placebo.**

Holmes et al 2001 **- 21 people with agitation, lavender, cross-over**

Smallwood et al 2001 **– 21 people, lavender, randomised comparison massage**

Ballard et al 2002- 72 **people with agitation, *Melissa,* double blind placebo controlled**

Lee et al 2005 **- Lavender versus jojoba massage**

Lai et al 2007 **- 70 people Lavender versus sunflower oil inhalation**

SIGNIFICANT REDUCTION IN AGITATION/ AGGRESSION

PHARMACOLOGICAL DISSECTION ESSENTIAL OILS

- **Essential oils** *Melissa officinalis (lemon balm)* and *Lavendula angustifolia (lavender) have* calming and sedative properties.
- Both essential oils (EO) may help in agitation
- Melissa may also increase attention (pro-cognitive)
- 3 trials: BMJ Editorial suggests improvement in behaviour, quality of life, as well as social and constructive activities

Burns et al (2002) BMJ;325(7376):1312-3

Ion Channels Investigated to probe mechanism

- $GABA_A$ receptor

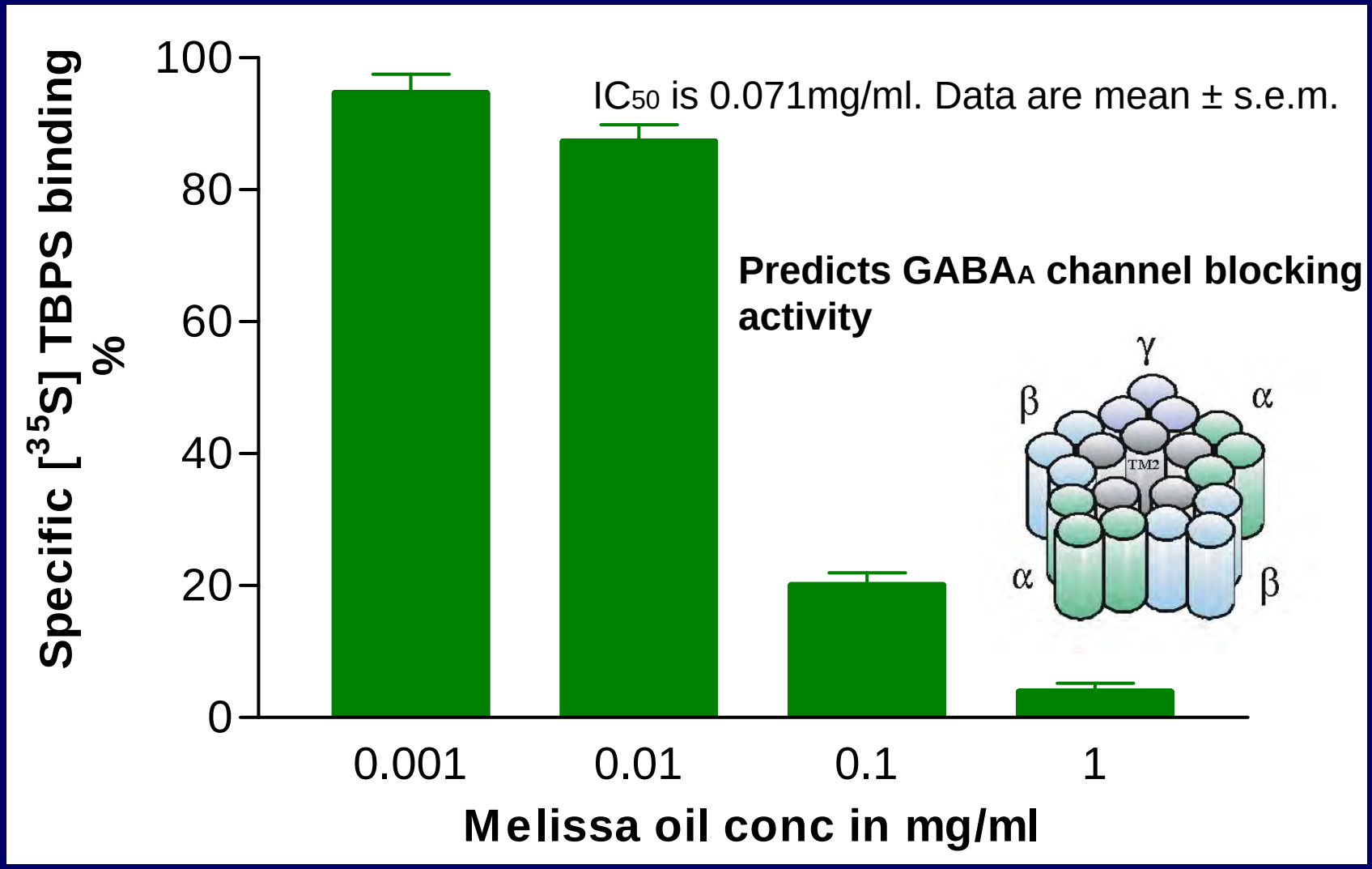
major inhibitory receptor in the brain

- Voltage-Gated Sodium Channel

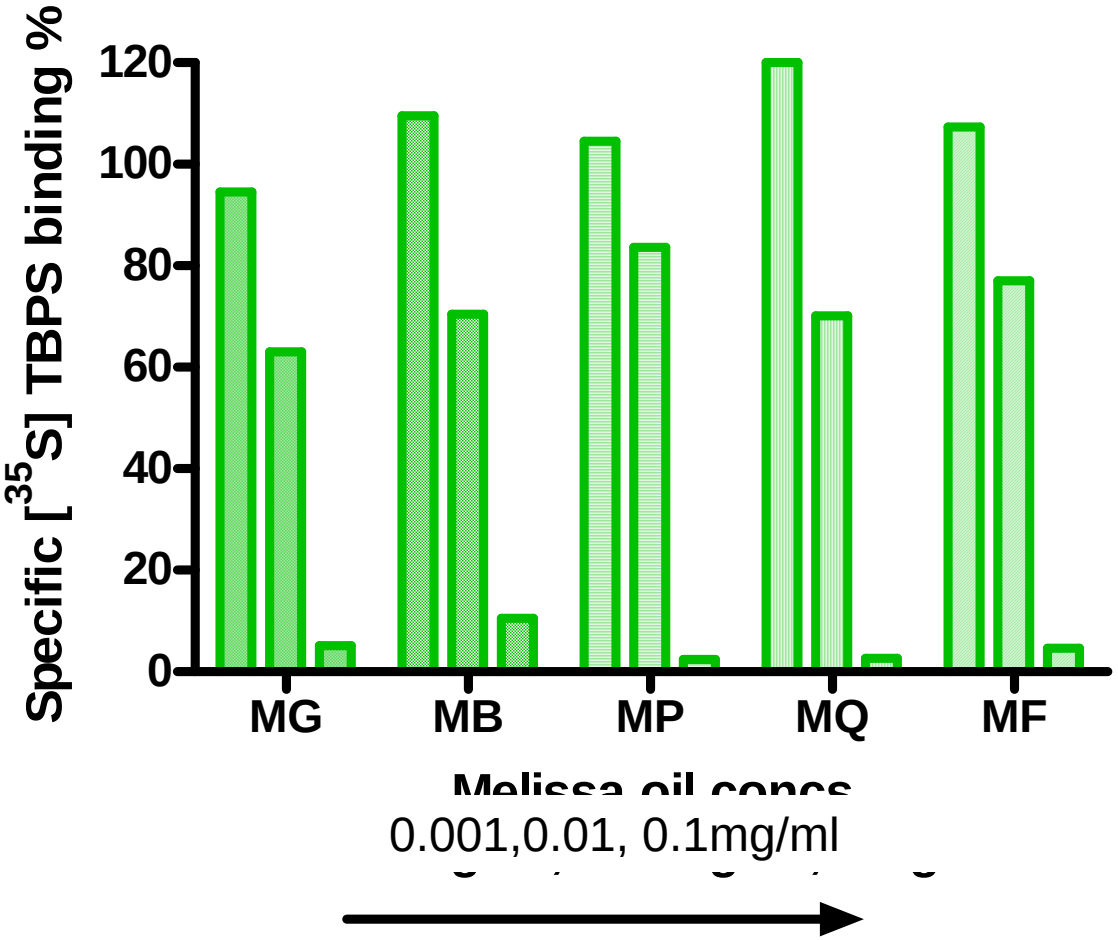
fundamental for neurotransmission

Radioligand binding pharmacological screens

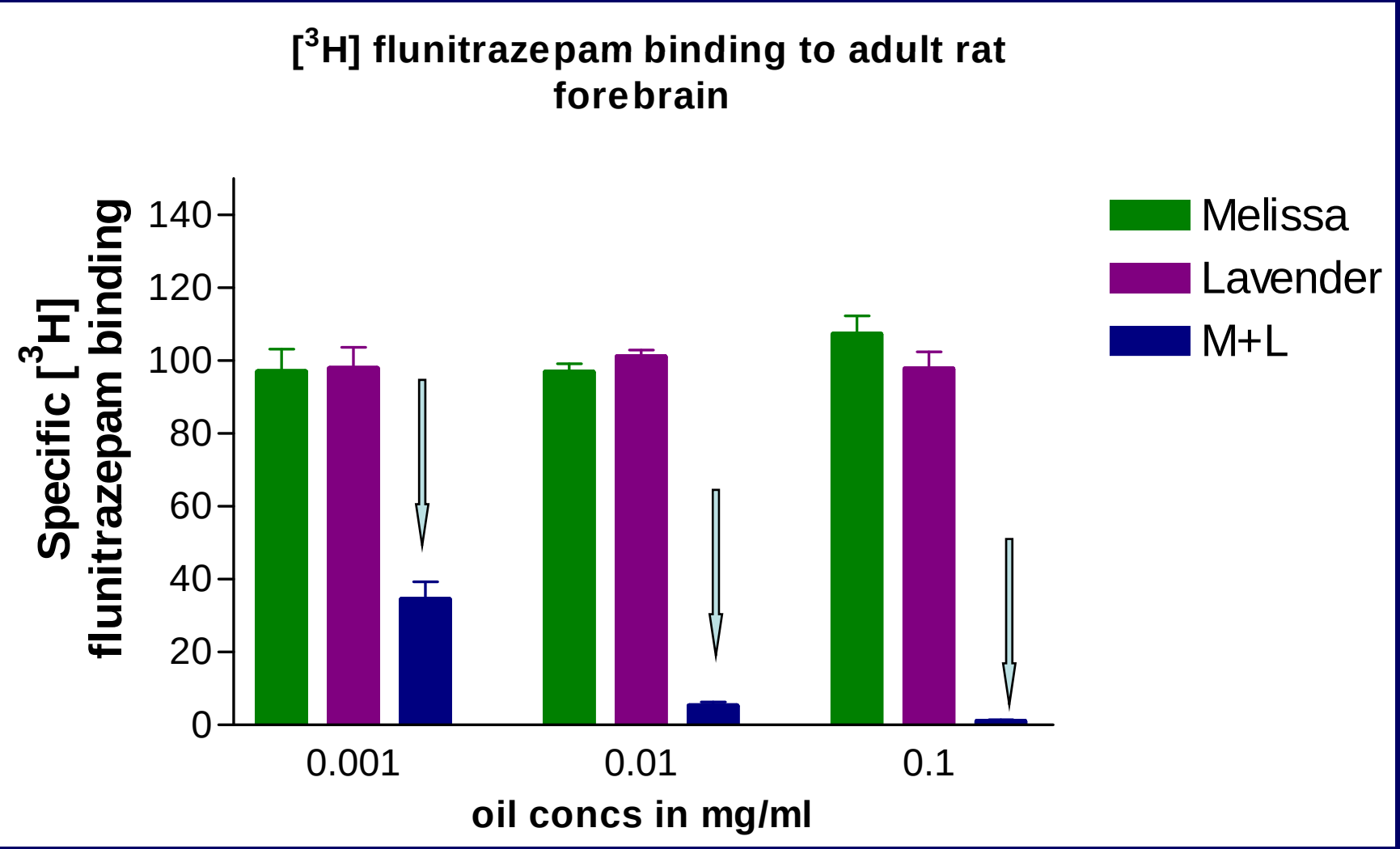
Both oils contain a GABA-A receptor blocker



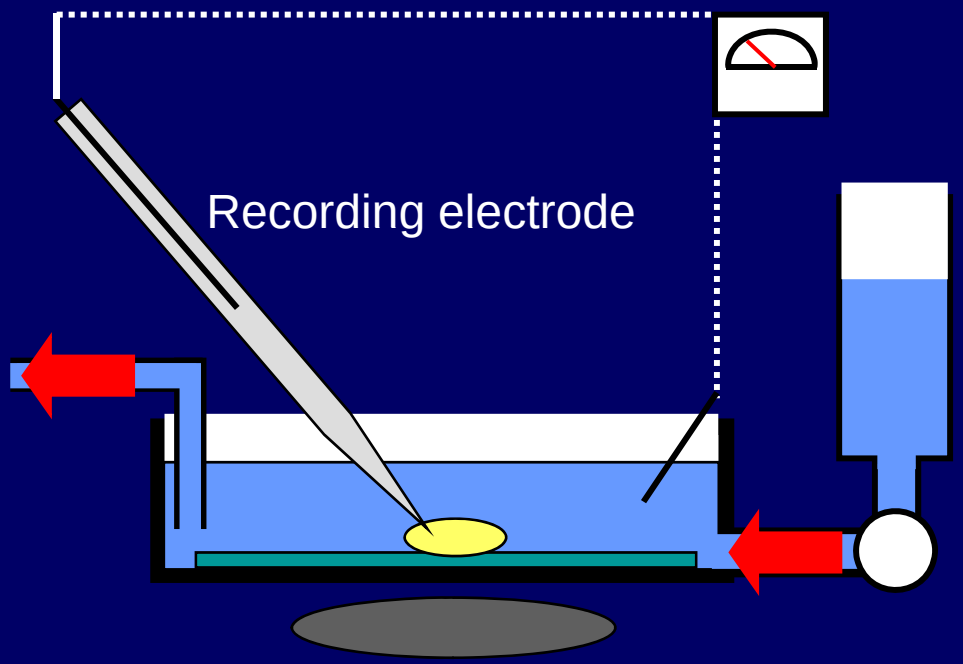
Melissa oil Batch supplier comparison

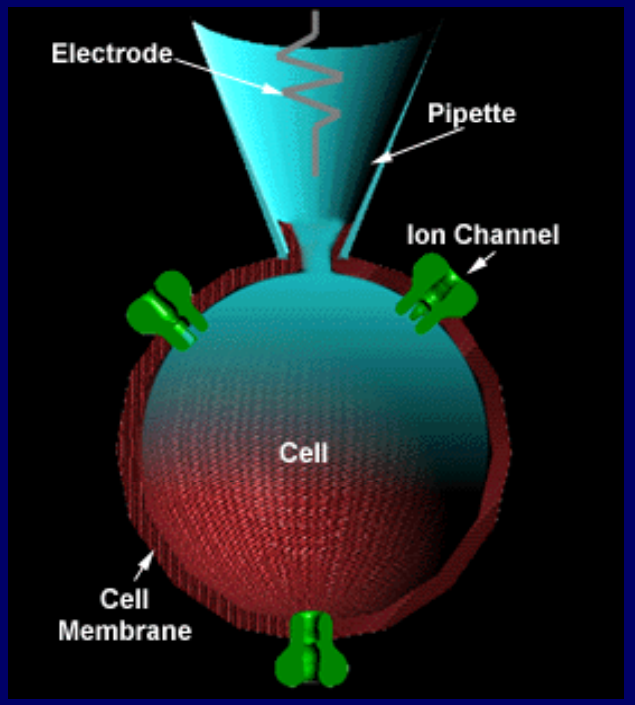


Melissa & Lavender EO Combination effect

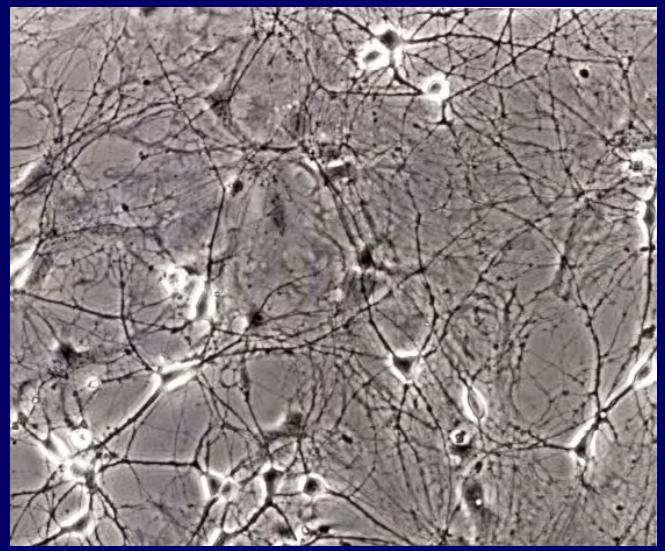


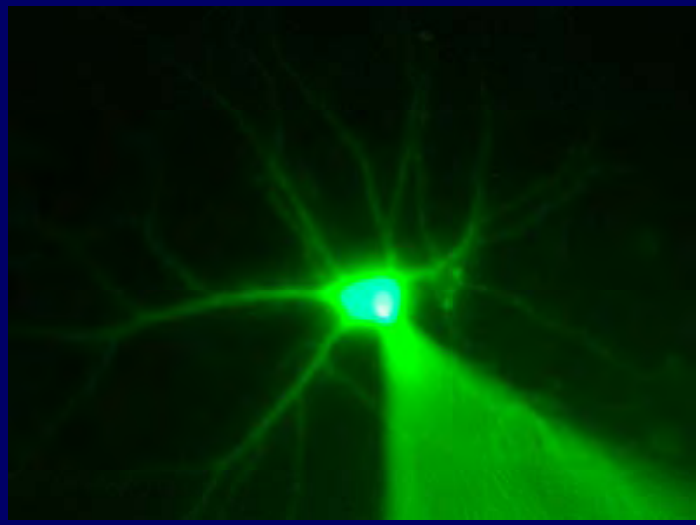
Electrophysiology



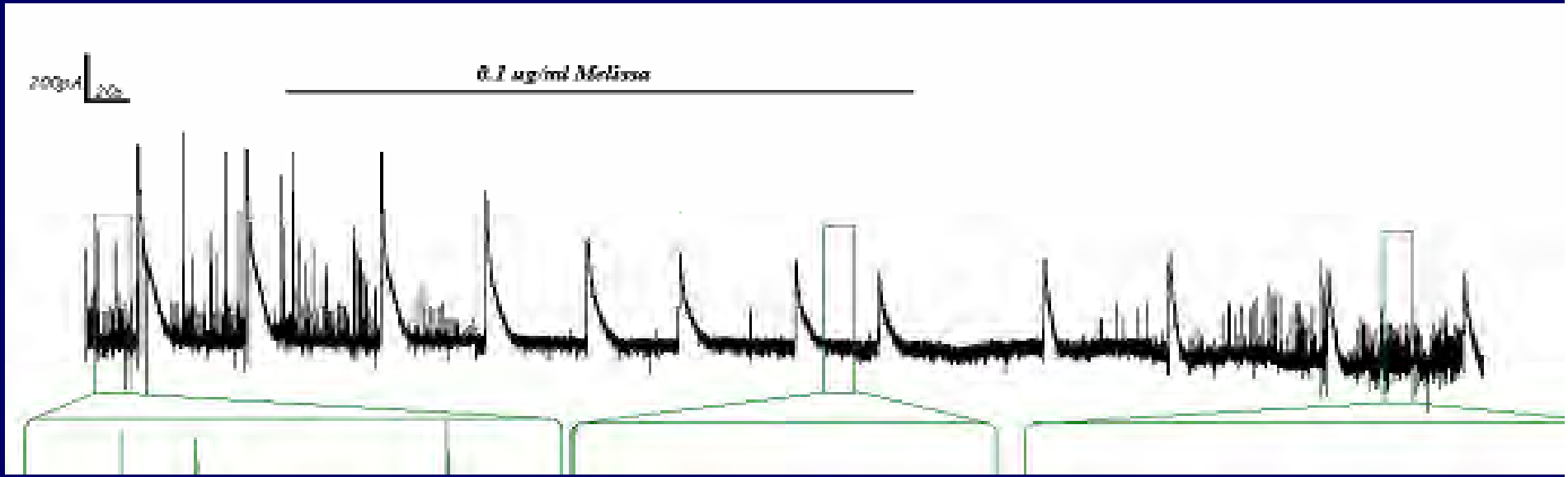


Primary rat cortical cultures
DIV 21-28



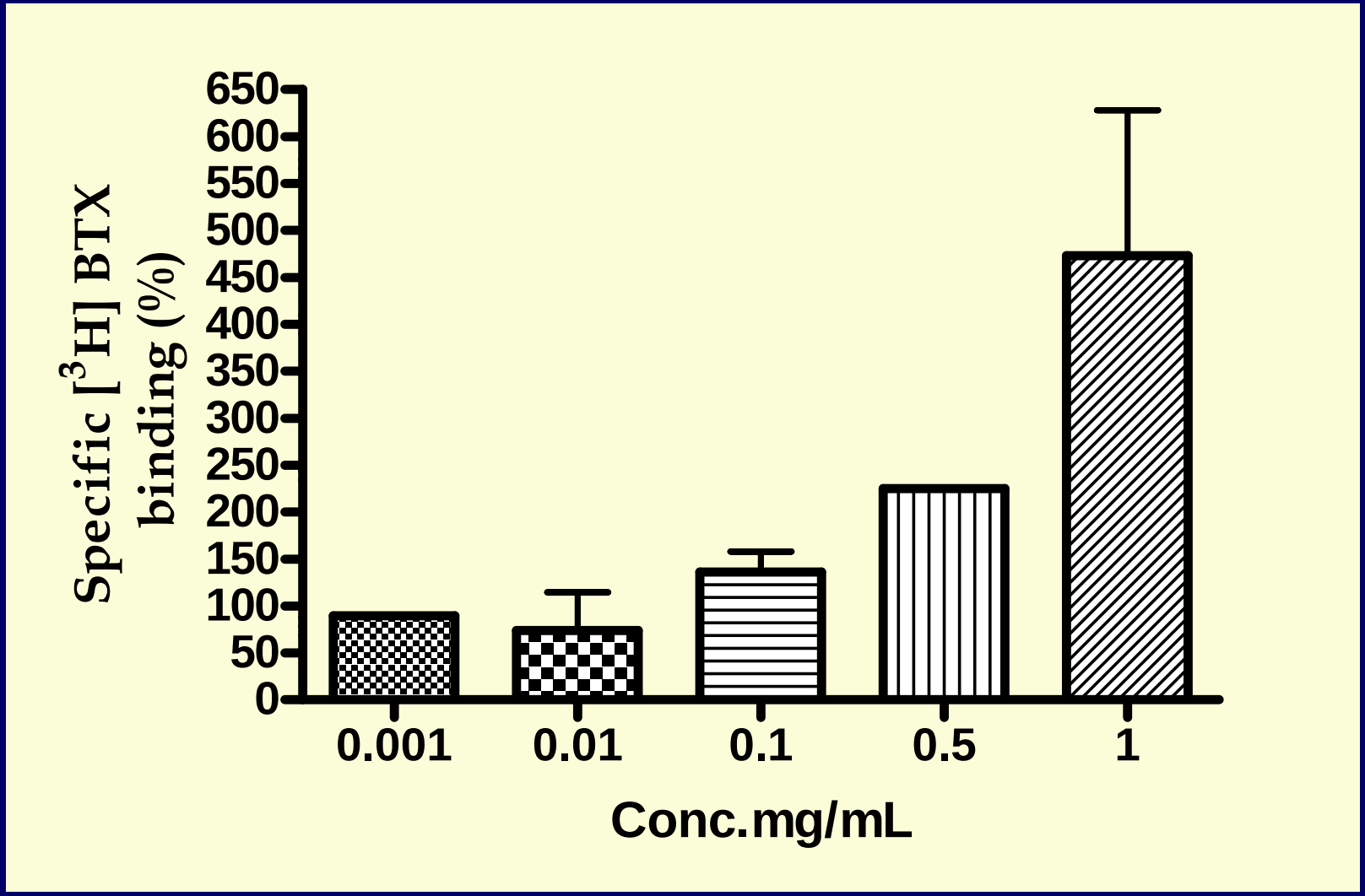


Both oils completely blocked evoked GABA inhibitory currents

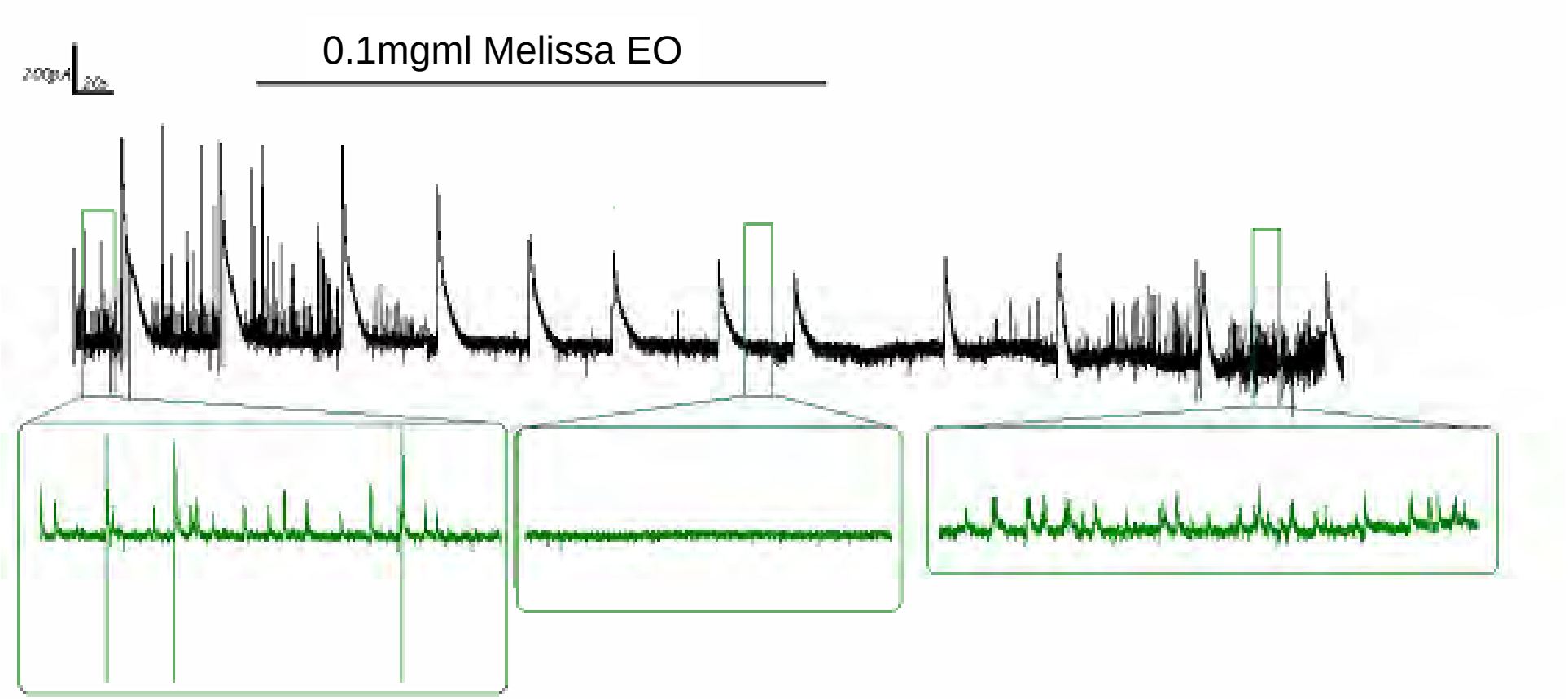


Effect of MO on GABA-mediated currents in primary cortical neurons.

Both EOs contain a sodium channel modulator



Both oils are profoundly depressant despite disinhibition

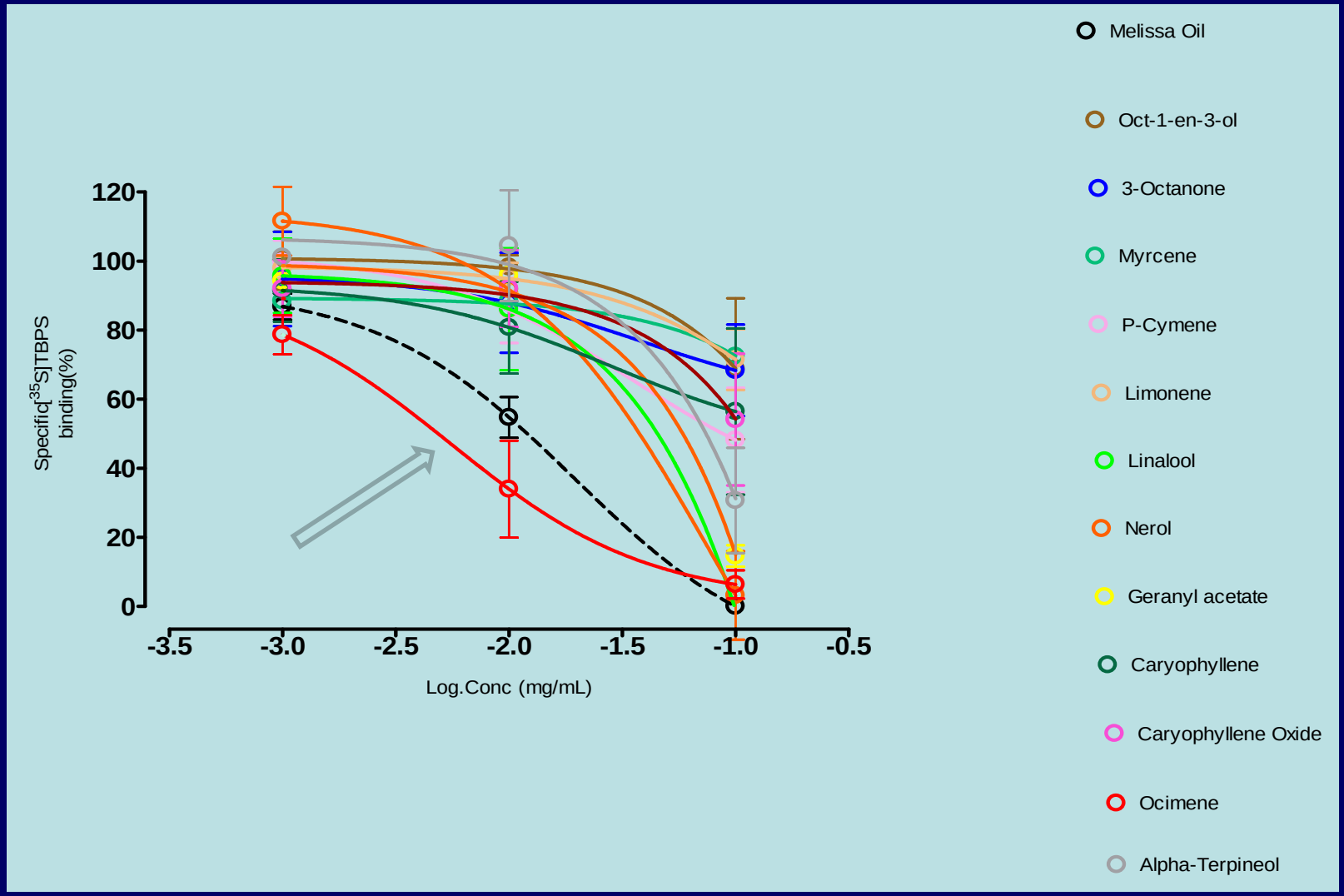


Compounds detected in essential oils from both *Lavandula angustifolia* and *Melissa*, obtained from GC-MS

Compound	*M.offinalis* Oil Composition (%)[a]	*M.offinalis* Oil Composition (%)[b]	*L.angustifolia* Oil Composition (%)[a]	*L.angustifolia* Oil Composition (%)[b]
Oct-1-en-3-ol	0.6	0.6	0.2	0.2
3-Octanone	tr	tr	0.3	tr
Myrcene	0.1	tr	0.5	0.4
p-Cymene	tr	tr	0.2	0.3
Limonene	tr	tr	0.3	0.2
(E)-β-Ocimene	0.5	0.6	1.3	1.6
Linalool	0.8	0.8	30.8	31.1
α-Terpineol	0.1	tr*	1.3	1.7*
Nerol	0.9	1.1	0.2	0.3
Geranyl acetate	3.3	3.6	1.0	1.1
(E)-Caryophyllene	12.3	12.9	3.6	3.6
Caryophyllene oxide	3.9	3.7	0.6	0.7

[a]Chromatography performed on a DB-5MS phase ;[b]chromatography performed on a ZB-WAX phase ;tr: < 0.1 % ;
* co-elution of α-terpineol and germacrene D.

Identification of new GABA$_A$ receptor antagonist



The effects of Melissa officinalis essential oil constituents on TBPS binding to well-washed rat forebrain membranes. All data are expressed as the mean ± SEM from at least three separate experiments.

Effects of Melissa oil constituents on the channel binding site of the $GABA_AR$ labelled by [^{35}S] TBPS

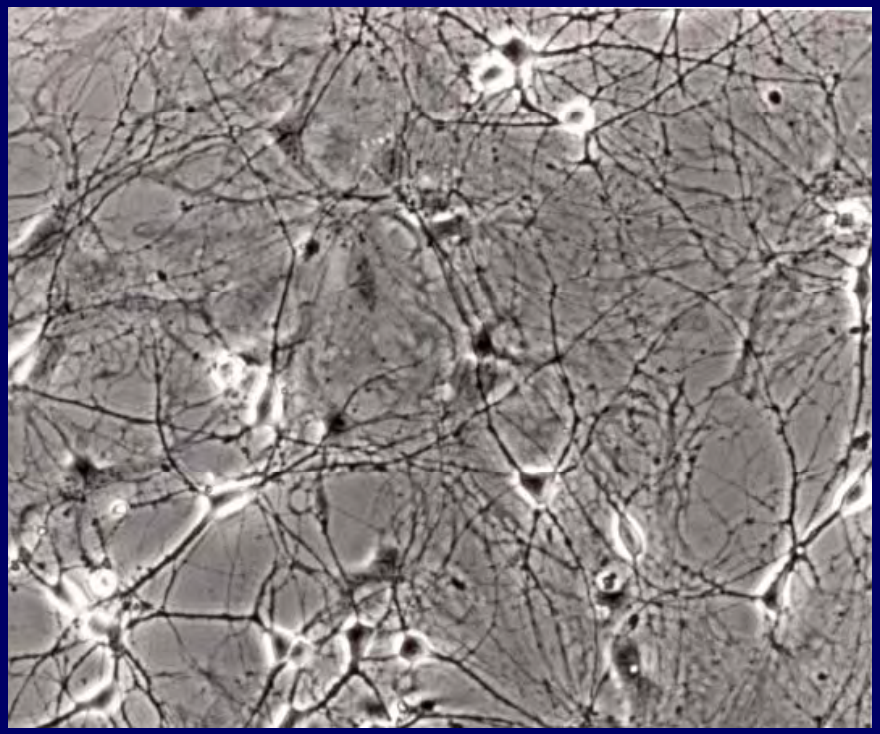
Chemical name	Molecular formula	IC_{50}(mg/ml)	IC_{50}(μM)
Melissa Oil	-	0.019	-
Geranyl acetate	$C_{12}H_2OO_2$	17.11	87166.9
(E)-Caryophyllene	$C_{15}H_{24}$	0.028	137.7
Caryophyllene oxide	$C_{15}H_{24}O$	0.584	2650.3
Limonene	$C_{10}H_{16}$	0.256	1878.3
Myrcene	$C_{10}H_{16}$	6.840	50205.5
Ocimene*	$C_{10}H_{16}$	0.006	40.5
p-Cymene	$CH_3C_6H_4CH(CH_3)_2$	0.035	261.6
3-Octanone	$C_8H_{16}O$	0.038	298.6
Linalool	$C_{10}H_{18}O$	0.900	5836.6
Nerol	$C_{10}H_{18}O$	0.079	513.3
Oct-1-en-3-ol	$C_8H_{16}O$	1.140	8890.9
α-Terpineol	$C_{10}H_{18}O$	0.211	1369.2

Effects of Melissa oil constituents on the sodium channel binding labelled by [^{3}H] BTX-B

Chemical name	Molecular formula	EC_{50}(mg/mL)	EC_{50}(μM)
Geranyl acetate	$C_{12}H_2OO_2$	>1	-
(E)-Caryophyllene	$C_{15}H_{24}$	>1	-
Caryophyllene oxide	$C_{15}H_{24}O$	>1	-
Limonene	$C_{10}H_{16}$	>1	-
Myrcene	$C_{10}H_{16}$	>1	-
Ocimene	$C_{10}H_{16}$	>0.5	-
p-Cymene	$CH_3C_6H_4CH(CH_3)_2$	>1	-
3-Octanone	$C_8H_{16}O$	>1	-
Linalool	$C_{10}H_{18}O$	>1	-
Nerol	$C_{10}H_{18}O$	>1	-
Oct-1-en-3-ol	$C_8H_{16}O$	>1	-
A-Terpineol	$C_{10}H_{18}O$	>1	-

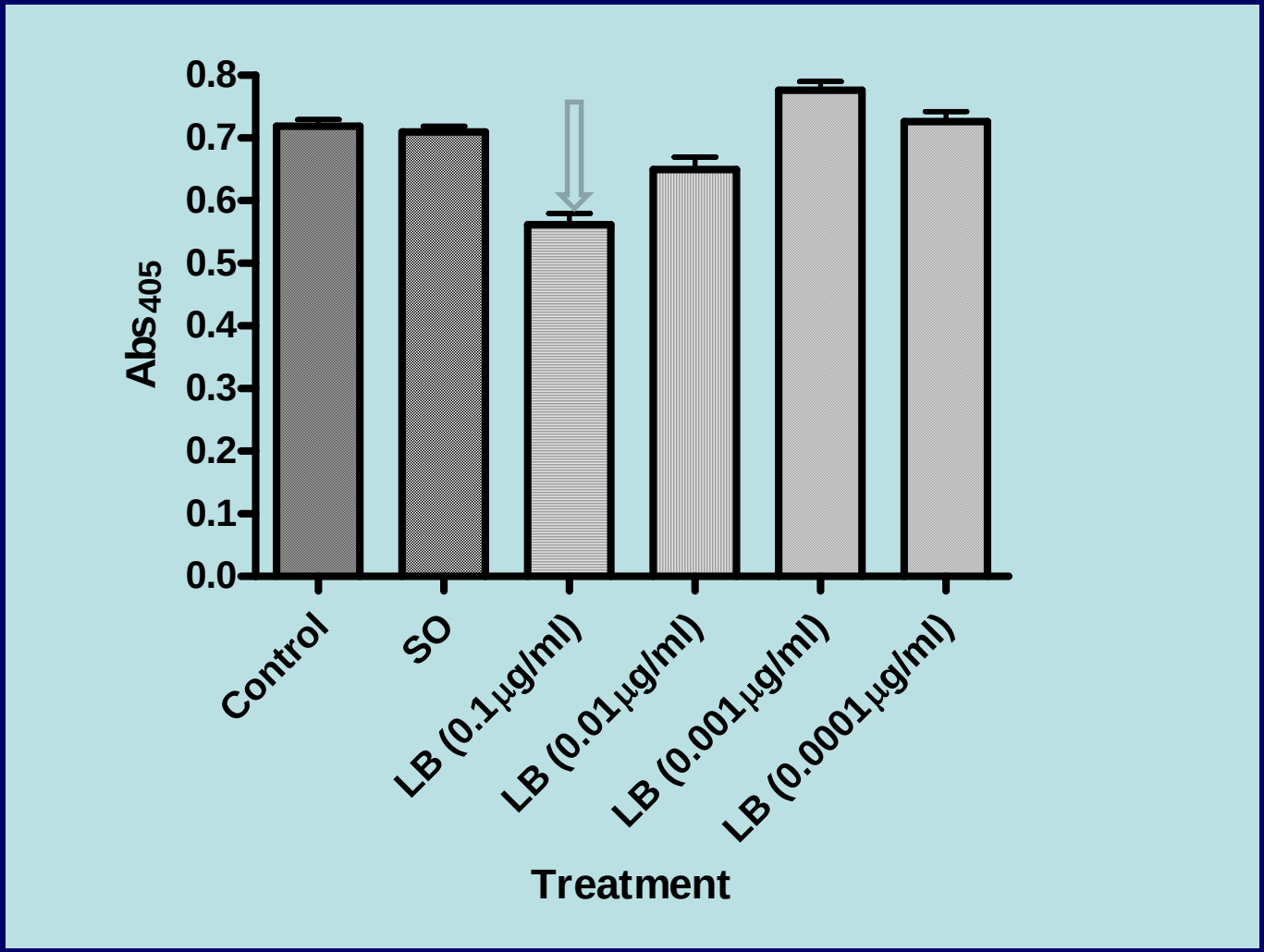
- No potent effects (< 0.5 mg/ml) of major components upon [^{3}H] BTX binding
- The sodium channel modulator is likely to be a minor component
- Fractionation and more sensitive detection methods required

Biochemical pharmacology screen



Sunflower oil (control) v Melissa essential oil

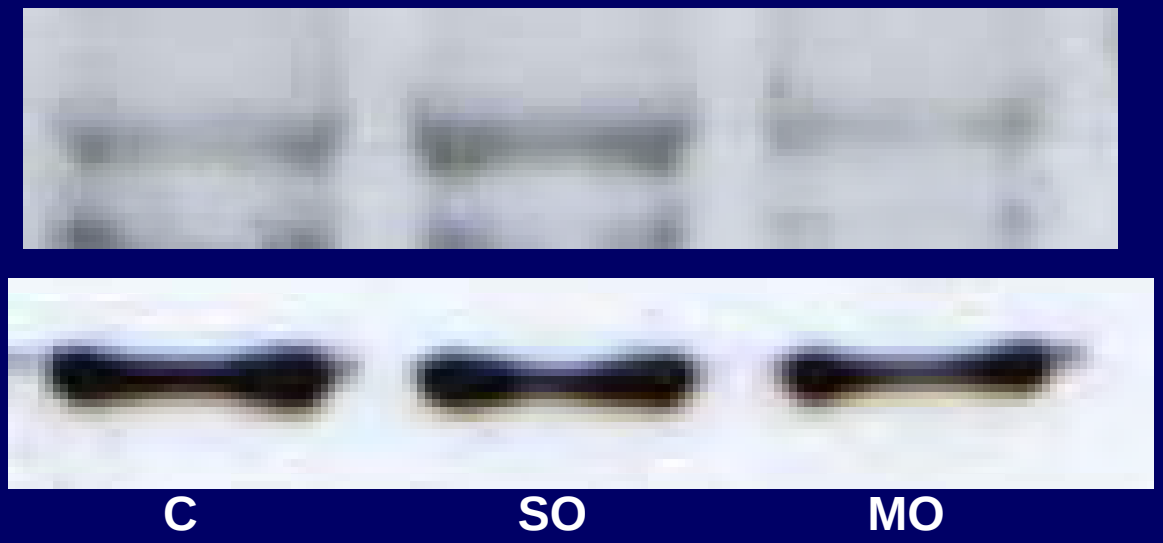
Melissa oil is mildly neurotoxic at high concentrations



Mild toxicity at high concentrations consistent with low affinity $GABA_AR$ inhibitory properties/modest protection at 0.001mg/ml

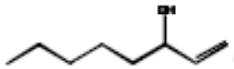
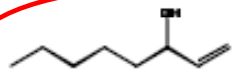
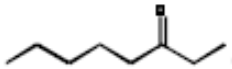
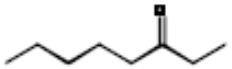
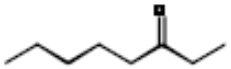
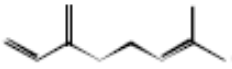
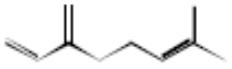
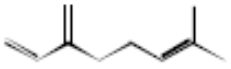
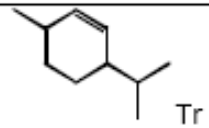
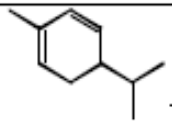
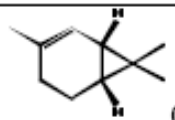
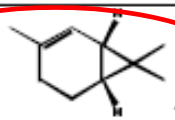
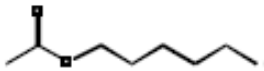
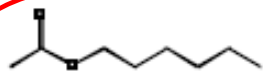
Dominating neuronal depressant activity

Effect of MO (24h exposure) on *cfos* expression



Exposure to MO (0.01 mg/ml)
reduces *cfos* expression (marker of neuronal activation

Approach to separate the various pharmacological components: Solid Phase Fractionation of Essential oils

	Whole	Fraction 1	Fraction 2	Fraction 3
1-Octen-3-ol	 0.4	0	Tr	 1.4
3-Octanone	 0.1	 0.1	 0.1	Tr
Myrcene	 0.5	 2.6	 0.1	Tr
a -Phellandrene	 Tr	 Tr	0	0
2-d-Carene	 0.3	 2.1	0	0
Hexyl acetate	 0.1	Tr	 0.1	0

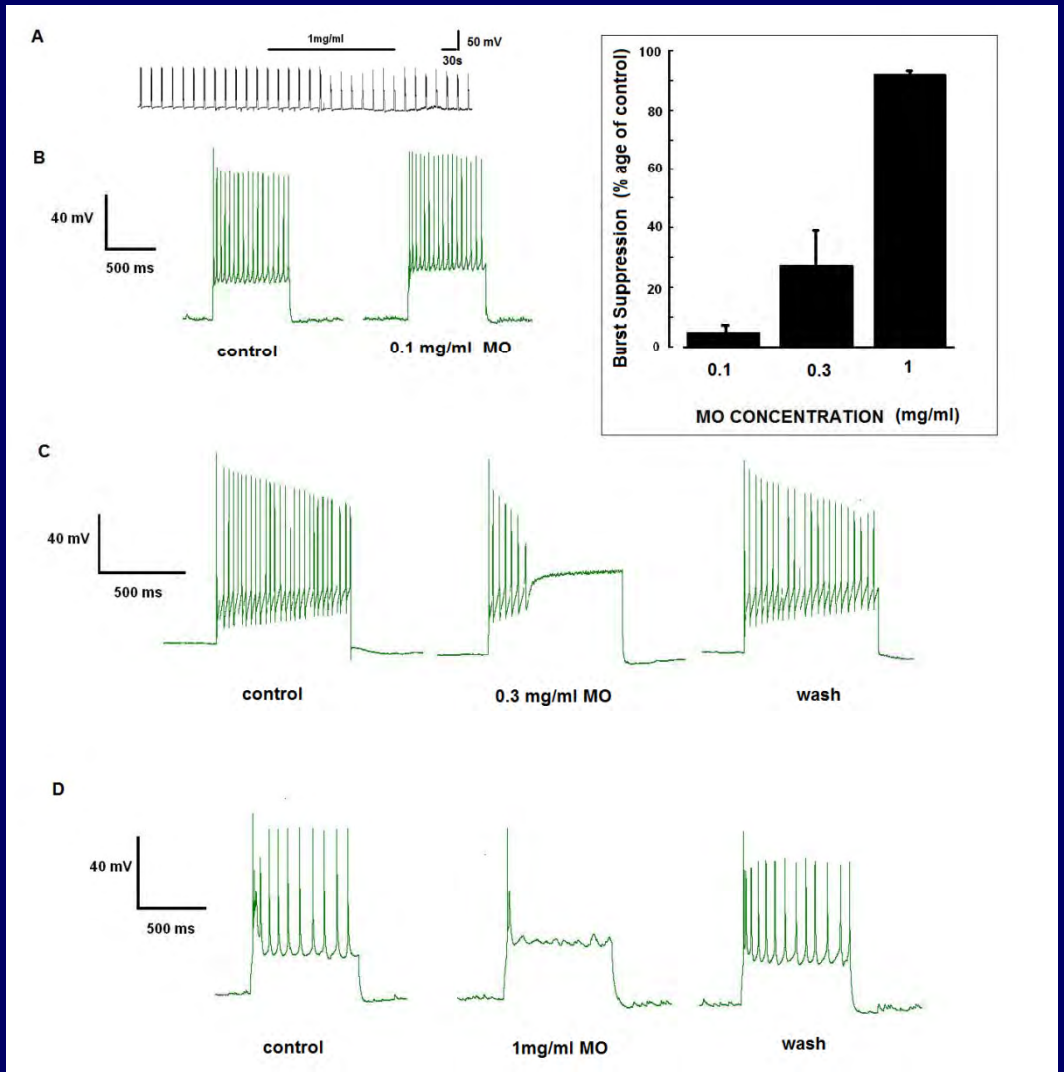
Hydrocarbons (incl. ocimene

Carbonyls
Ethers
esters

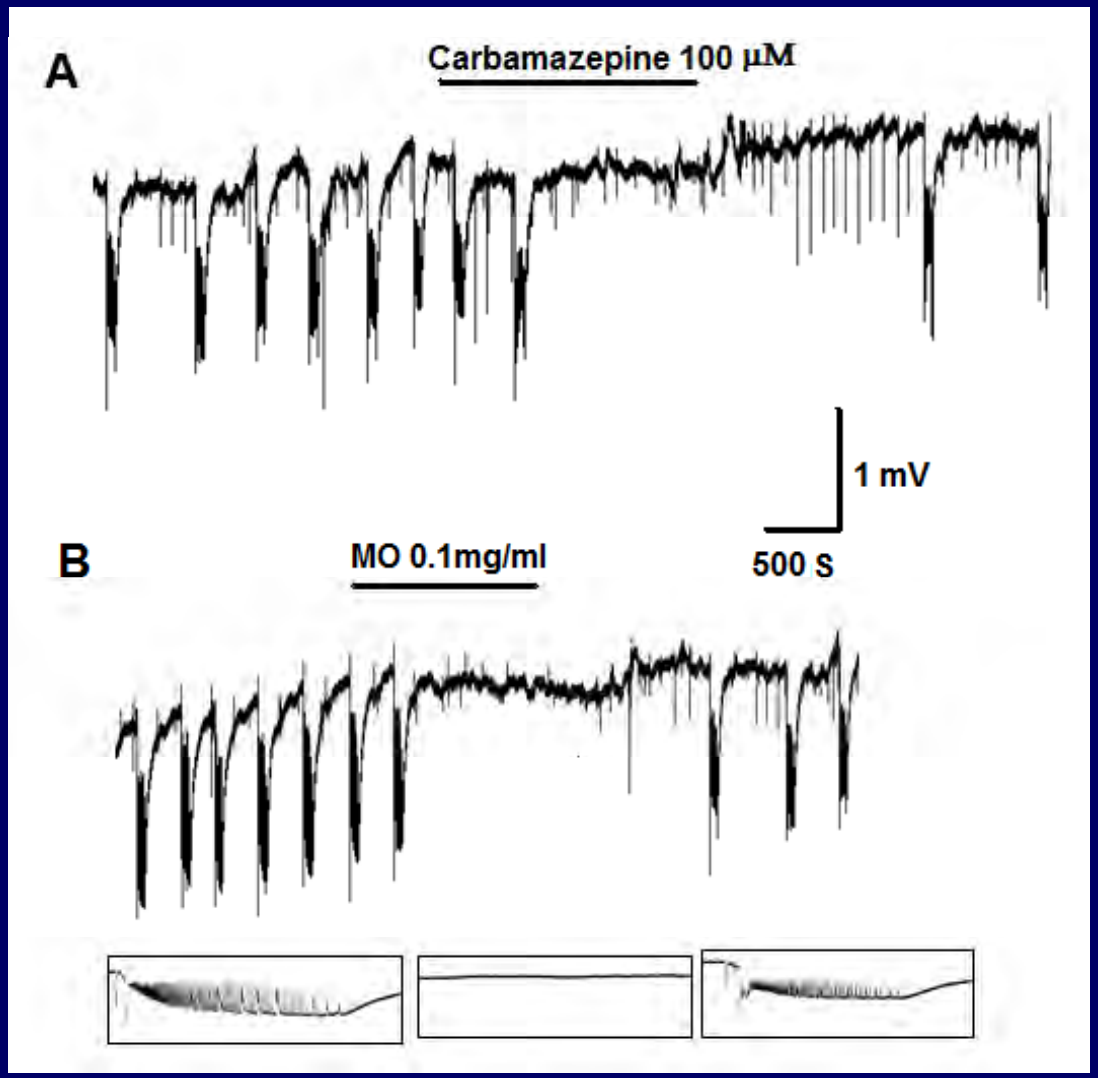
Primary alcohols
Diols
Acids

New Potential of Melissa in epilepsy and hyperalgesia

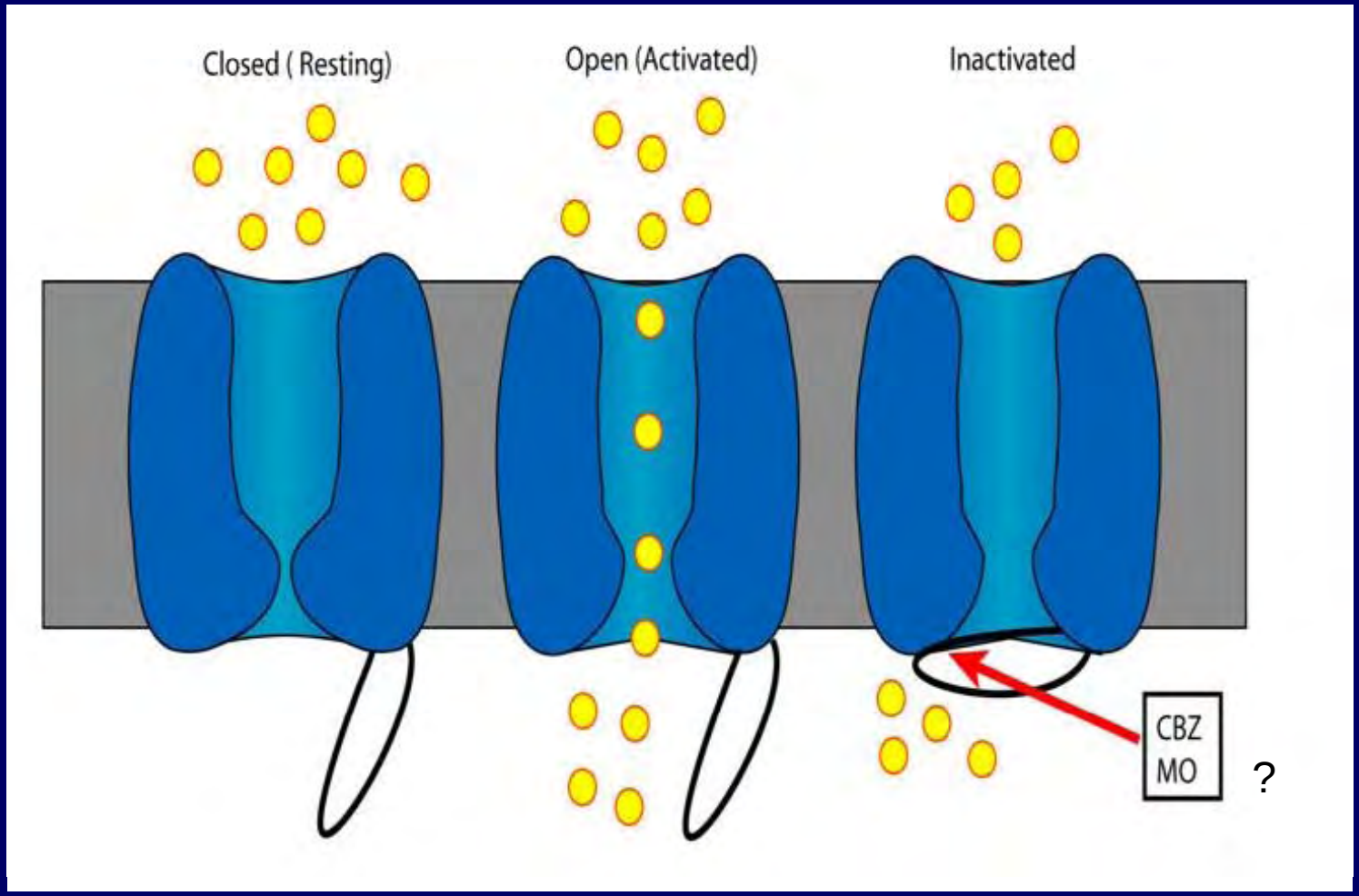
Reversible depressant effects of MO on evoked bursts of action potentials & Concentration-dependent suppression of the secondary spikes in the burst profile



MO can reversibly reverse ictal-like epileptiform activity in a 4-AP-induced brain slice (visual cortex) model for epilepsy



The modulated site for anticonvulsants on the VGSC complex



Melissa and Lavender EO Pharmacological dissection

- The volatile oils contain a novel $GABA_A$ receptor antagonist lead compound, namely ocimene
- The dominant net effect of the essential oils (lavender & Melissa) on neurons is depressant
- The mechanism for depressant defined as inhibition of membrane excitability/electrogenesis via a VGSC
- Fractionation protocols & computer modelling strategies under development for identifying VGSC modulators
- We have identified novel therapeutic arenas for use of Melissa and Lavender essential oils, namely epilepsy & hyperalgesia

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