

Research paper

Effects of camphor and related compounds on slowly adapting mechanoreceptors in the rat sinus hair follicle

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ABSTRACT

Introduction: Camphor is a popular compound for therapeutic and cosmetic use with a distinctive odour, and somatosensory warming and cooling properties. The mechanisms for its action remain unclear.

Objective: The current study examined the effects of two enantiomers of camphor and related monoterpenoid compounds on mechanoreceptors.

Methods: Extracellular recordings were made in an *in vitro* bath preparation. Camphor, borneol, eugenol, carveol, and thymol were tested on the neural activity of St I and St II slowly adapting mechanoreceptors in the rat vibrissal hair follicle preparation.

Results: All compounds tested (0.5 – 2 mM bath concentrations) resulted in dose-dependent depression of spontaneous and mechanically evoked firing (dynamic and static phases). The mean latency of responses also increased. Both St I and St II were similarly affected, although (-)-camphor had a greater depressant effect on St II than on St I units. Differences were found across the different compounds for their effect on the dynamic and static phases. Thymol was found to have the greatest depressant effect on these phases. The broad spectrum TRP blocker ruthenium red did not reverse the depressant effects of camphor. The depressant effects of the compounds appeared similar to those obtained using the local anaesthetic lignocaine. The depressant effects of camphor and of lignocaine were partially reversed by the K⁺ channel blocker tetraethylammonium.

Conclusions: The results question whether the depressant effects of camphor and related compounds act through TRP channels. Perhaps the use of more selective blockers may reveal the molecular mechanisms through which these compounds act.

Introduction

There is great interest in the biomolecular mechanisms that underlie the actions of camphor and camphor-related monoterpenoid compounds because of their wide use in cosmetics, health and medicine (Chen et al., 2013), although its wide use has not been without dangers (Uc et al., 2000; Love et al., 2004). As well as its distinctive aroma, it has both warming and cooling effects on the skin and mucous membranes. Camphor is a common constituent found in many topical preparations for its antipruritic, analgesic and anaesthetic effects (Hamidpour et al., 2013; Zuccarini, 2009). It has also been used as a perfume in the Middle East and as flavouring ingredient in bread and sweets. Camphor was originally obtained by distillation of camphor tree bark, *Cinnamomum camphora* (Lee et al., 2022). It is now produced synthetically from

turpentine oil and known chemically as 2-Camphanone and 2-Bornanone, with chemical formula C₁₀H₁₆O (Malabadi et al., 2021). It belongs to terpenoid class of organic chemicals. The cyclic terpene structure makes it highly lipophilic, which facilitates its movement across mucous membranes and leads to its large volume of distribution. Eugenol is a related compound, an allyl chain-substituted guaiacol, extracted from certain essential oils especially from clove, nutmeg, cinnamon, basil and bay leaf (Prasad et al., 2010), while borneol is a precursor to camphor (produced through oxidation) (Chen et al., 2013). Other monoterpenoids, carveol and thymol, are used as fragrances and in traditional medicine (Bhatia et al., 2008; Parsaei et al., 2016).

The olfactory properties of camphor have been studied (Fesenko et al., 1979; Duchamp-Viret et al., 1999; Sicard, 1985), but more extensive studies have been made of its somatosensory properties,

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particularly for the prominent sensations of cooling and warming. Camphor appears to potentiate the sensations of both warming and cooling stimuli applied to the hairy skin (Green, 1990). A study using camphor, eucalyptus and menthol found that they evoked cooling sensations in the nasal passages, but without affecting airflow (Burrow et al., 1983). Camphor between 5% and 20% in petroleum jelly applied to the medial skin of the forearm produced initially cooling effects which were superseded by a warming sensation. The same application of menthol produced a non-adapting cooling effect. Both menthol and camphor increased local blood flow in the skin (Kotaka et al., 2014).

A number of transient receptor potential (TRP) channels have been associated with mechanosensory, nociceptive, and thermal transduction processes (Ramsey et al., 2006; Lumpkin and Caterina, 2007). Camphor modulates the activity of temperature-activated ion channels in the TRP family. It acts as an agonist at warmth-sensitive TRPV3 channels (Moqrich et al., 2005), partial agonist at heat-sensitive TRPV1 (Xu et al., 2005) and an inhibitor at the noxious cold sensor TRPA1 (Xu et al., 2005; Story et al., 2003; Macpherson et al., 2006). In HEK293 cells expressing human and rat TRPM8, camphor evoked calcium transients were blocked by TRPM8 antagonists, and camphor also sensitized cold-induced calcium transients and evoked desensitising outward-rectifying currents in these cells (Selescu et al., 2013). Actions at other receptors, acetylcholine and GABA_A (Park et al., 2001; Hall et al., 2004), are unlikely to explain their sensory effects. Camphor has actions in insects, activating the heat-sensitive TRPA1 homolog, the honey bee Hymenoptera-specific TRPA channel (Kohno et al., 2010), and partially blocks the heat-sensitive current of the *Drosophila* TRP channel Painless (Sokabe et al., 2008).

The two types of slowly adapting mechanoreceptors present in mammalian skin are functionally and anatomically distinct (Iggo, 1968). They both signal sustained tissue displacement to the central nervous system, though type I mechanoreceptors have small receptive fields (such as on the finger tips), while type II mechanoreceptors have larger receptive fields and may be more important in signalling proprioceptive information (Vallbo and Johansson, 1984). Slowly adapting type I mechanoreceptors are identified with Merkel nerve endings (Iggo and Muir, 1969), and it has been suggested that these cells associated with the nerve endings are themselves mechanotransducers, and transmit chemically to the nerve endings, possibly using glutamate (Fagan and Cahusac, 2001; Maksimovic et al., 2013).

A previous study in our laboratory found that (+)-camphor had depressant effects on all the response components type II slowly adapting mechanoreceptors in the rat whisker hair follicle (Cahusac, 2009). The principal hypothesis tested by the current study is that the effect of camphor would have differential effects on type I versus type II slowly adapting mechanoreceptors. A secondary hypothesis is that the two enantiomers of camphor (Malabadi et al., 2021) had different effects on these mechanoreceptors. A further hypothesis is that related monoterpenes (eugenol, borneol, thymol and carveol) would have similar effects, although there may be differences according to the class (monocyclic versus bicyclic). Previously, a study had found that these mechanoreceptors, more often type II units, were exquisitely sensitive to cooling and warming (Cahusac and Noyce, 2007). Rapidly adapting mechanoreceptors are much less sensitive (Iggo and Muir, 1969; Tapper, 1965; Iggo, 1969; Chambers et al., 1972). Studying the effects of these monoterpenoids on slowly adapting mechanoreceptors could provide biomolecular insights into the mechanisms that underlie the action of these compounds on sensory perception. The *in vitro* isolated sinus hair preparation allows prolonged single unit electrical recordings with greater stability than *in vivo* methods (Cahusac et al., 2005; Sonekatsu et al., 2022). We use the terminology of sinus type I and sinus type II (St I and St II) which refers to slowly adapting SA1 and SA2 respectively. This is the standard notation that was introduced by Gottschaldt et al. in 1973 (Gottschaldt et al., 1973) and subsequently adopted by many (though not all, e.g (Sonekatsu et al., 2022).) successive authors (Fagan and Cahusac, 2001; Senok and Baumann, 1997; Cahusac and Mavulati,

2009).

Methods

The Ethics Committee of the Stirling University Department of Psychology approved the use of animals in this study. All experimental procedures complied with UK Home Office regulations as well as the European Directive 86/609/EEC. The minimum number of animals was used in order to demonstrate statistically clear effects. Use of the Home Office Schedule 1 kill of animals ensured minimal suffering.

A total of 17 adult male albino Wistar-derived rats (mean weight 241 g, normal weight for 6-week old animals) were used. Male animals were used in order to increase the homogeneity of the data and the statistical power (de Winter and Cahusac, 2014), although this is at the expense of generalisability. As previously described (Cahusac and Noyce, 2007) each animal was deeply anaesthetised with intraperitoneal urethane (2 g/kg) followed by euthanising intracardial urethane. Whisker pads were removed and individual sinus hair follicles microdissected out and kept in carbogenated (95% oxygen, 5% carbon dioxide) synthetic interstitial fluid (SIF) (Bretag, 1969). The hair follicle was slit lengthwise to allow easy access of drugs the mechanoreceptors (Baumann et al., 1996; Cahusac and Senok, 2020), and pinned open on a Sylgard platform within a custom-made tissue bath (provided courtesy of Professor K. Baumann, Hamburg) as previously described (Baumann et al., 1996) and shown diagrammatically in Fig. 1 of that publication. The vibrissa was trimmed to 5 mm length and attached to a glass tube fixed to piezoelectric trimorph for mechanical ramp stimulation. A 10 mm length of the deep vibrissal nerve remained attached to the follicle. The nerve bundle was stripped of its sheath and split repeatedly until fine nerve strands could be isolated. A silver recording wire was immersed in Fluorinert (FC-40, Sigma) at the bottom of the bath. Carbogenated SIF was perfused over the follicle above the Fluorinert. Bath temperature was maintained between 29 and 33 °C, but restricted to ± 1 °C from the mean temperature for a given experiment. Extracellular electrical activity was recorded from nerve strands attached to the recording wire,

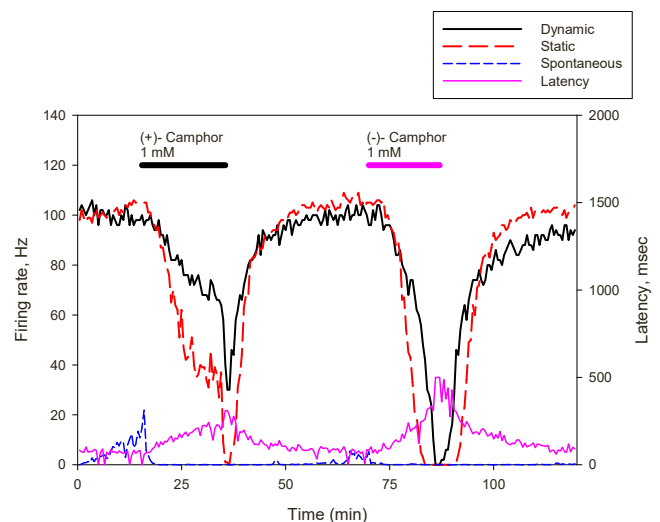


Fig. 1. The effect of the same dose of (+)- and (-)-camphor on an St II unit. The bars above the traces show the onset of the applications, each lasting 20 min. The first application ((+)-camphor 1 mM) produced a submaximal depression of evoked (dynamic and static phase) firing. After recovery, (-)-camphor (1 mM) produced a larger and longer lasting depressant effect. The latency of response increases during both of the applications, though more to the second application. The key gives the line styles indicating the activity: thick continuous is dynamic phase, thick long-dashed is static, thin short-dashed is spontaneous, thin continuous is latency. The left vertical axis represents firing rate in spikes/sec (Hz) while the right axis represents the latency of onset of the first spike in the evoked response, measured in msec.

using Digitimer Neurolog equipment (Welwyn Garden City, UK), amplified and filtered, and monitored on oscilloscopes and through a loud speaker. Initial responsiveness and characterisation were achieved by deflecting the vibrissa using fine forceps, which also helped isolate individual units of interest. Trapezoid movement stimuli consisted of a 0.1 – 2 mm deflections, with 0.5 s onset, 4 s plateau and 0.5 s offset ramp. Stimuli were applied every 30 s. The activity of units was recorded for each ramp and between ramps. The dynamic phase of activity was during the ramp onset, while the static phase was during the last 1 s period of the plateau, and the spontaneous phase was between stimuli. The variability in firing during the static phase was used to identify the type of slowly adapting unit (St I or II). St I units had a coefficient of variation of inter-spike intervals that exceeded 0.1, while St II units had a coefficient of variation < 0.1 (Baumann et al., 1996). Plots of inter-spike intervals, and of interspike intervals against successive spikes (Iggo and Muir, 1969) were done during the experiment and were a useful indicator of the type of unit. Further confirmation of their unit type was obtained by the effects of 10 mM caffeine (Senok and Baumann, 1997) and 3 mM tetraethylammonium (TEA) (Senok et al., 2001). A custom-written SPIKE2 (Cambridge Electronic Design, Cambridge, UK) scripts were used to control, collect and analyse data on a computer. Specifically, the analysis scripts extracted the number of spikes that occurred during the dynamic phase (0 – 0.5 s from ramp onset to plateau), the static phase (the last 1 s of ramp plateau) and the number of spikes representing spontaneous firing during the 25 s interstimulus interval.

Stock solutions were made up of the following drugs: (+)-borneol (Aldrich) 1 M in DMSO, (+)-camphor (Fluka) 1 M in DMSO, (-)-camphor (Aldrich) 1 M in DMSO, (-)-carveol (Aldrich) 1 M in DMSO, eugenol (Aldrich) 1 M in DMSO, thymol (Sigma) 1 M in DMSO, tetraethylammonium (TEA) (synthesised by Dr E. Porter, Stirling) 50 mM in water, lignocaine (Sigma) 10 mM in water, ruthenium red (Sigma) 30 mM in DMSO. All drug solutions were diluted in 20 ml of SIF at pH 7.4. Concentrations of DMSO were 0.2% or less. Drug solutions were introduced into the bath at a rate of 1 ml/min, typically for 20 mins. The duration of drug effects was measured as the time taken from the end of the drug application (start of wash) until activity returned to $\pm 15\%$ of baseline values.

Parametric statistical tests (ANOVA, ANCOVA and *t* tests) were carried out using jamovi (Version 2.3.2, <https://www.jamovi.org>). Firing rates for each epoch of analysis were first averaged, and then the average effect of drugs expressed as % of control. The dose of drug given was calculated using

$$Dose = \ln(\text{Application Time}) \times \ln(\text{Drug concentration})$$

Results

A total of 22 single slowly adapting mechanoreceptor units were studied, 8 St I and 14 St II units. All of the compounds caused a decrease in firing, both spontaneous and mechanically evoked. An example of the effect of two enantiomers of camphor on a St II unit is shown in Fig. 1.

Figs. 2–4 illustrate that the effects of compounds were dose-dependent, higher concentrations had greater depressant effects.

A summary of the effects of all compounds tested is given in Table 1.

All the compounds tested tended to reduce the firing of the St units, affecting the spontaneous as well as the evoked firing (dynamic and static phases). Taking into account drug dose (see Methods) this was included as a covariate in an ANCOVA. The analysis only included those compounds for which there were sufficient data ($N > 8$), and so excluded eugenol and (+)-carveol. Each phase of activity was examined.

There were minimal differences between the compounds on spontaneous activity, $F(3,58) = 2.10$, $p = .110$, partial $\eta^2 = 0.10$. There was a marginally significant effect of differences between compounds on the static phase of activity, $F(3,58) = 2.77$, $p = .049$, partial $\eta^2 = 0.12$. For the dynamic phase, the analysis revealed clear differences between the

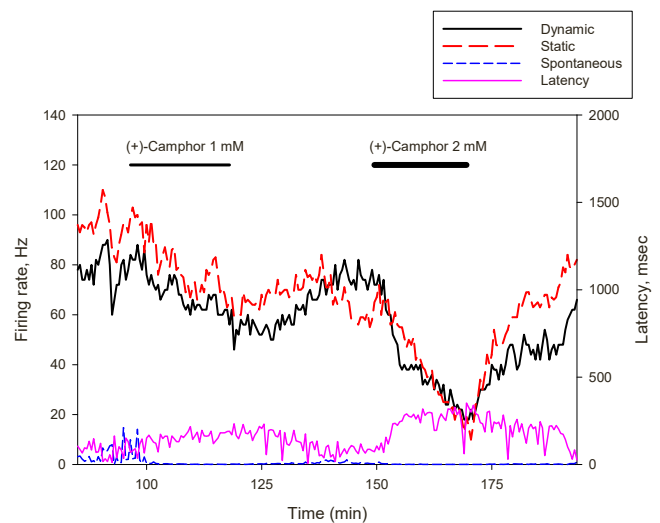


Fig. 2. Dose-dependent effect of (+)-camphor on the evoked firing of an St I unit. The lower dose of 1 mM had a minor effect on dynamic and spontaneous phase firing (it also silenced the low spontaneous firing). The higher dose of 2 mM had a much greater effect on the evoked activities. The latency of the response also increased markedly. All activities (except for the low spontaneous) recovered at the end of the experiment (right side of plot). Explanation for the axes and key are given in the Fig. 1 legend.

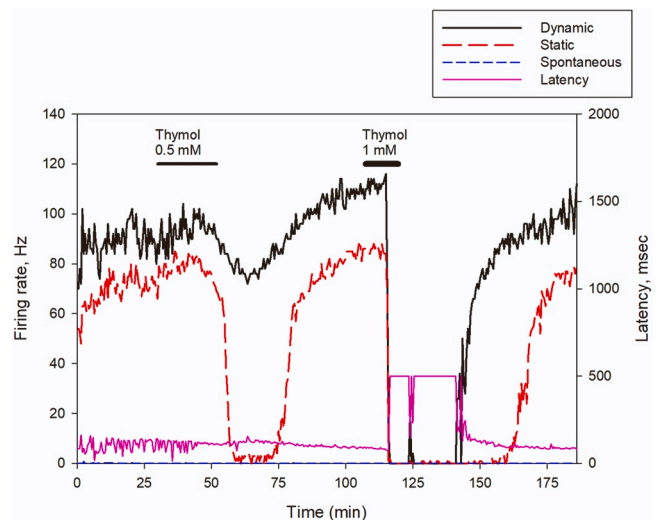


Fig. 3. The depressant effects of thymol applied at 0.5 and 1 mM to an St II unit. The lower dose had a marked effect on the static component, with little effect on the dynamic phase and the latency of response. Twice the concentration had much larger effects on all measures, with the dynamic phase response and latency recovering before the static phase response. Latency responses above 500 msec (the duration of the dynamic phase component) are assigned a value of 500 msec. Explanation for the axes and key are given in the Fig. 1 legend.

different compounds, $F(3,58) = 4.94$, $p = .004$, partial $\eta^2 = 0.20$. Post hoc analysis with Tukey HSD showed that thymol had the most potent depressant effects on the dynamic phase. A direct comparison of the effects of thymol versus eugenol is shown in Fig. 5. Finally, there were minimal differences between compounds for latency changes, $F(3,58) = 1.52$, $p = .219$, partial $\eta^2 = 0.08$.

Looking at the effects of all the compounds taking into account the dose of drug (covariate in ANCOVA), there was no difference between their effects on St I versus St II units. However, looking at individual compounds revealed that (-)-camphor had a greater depressant effect on

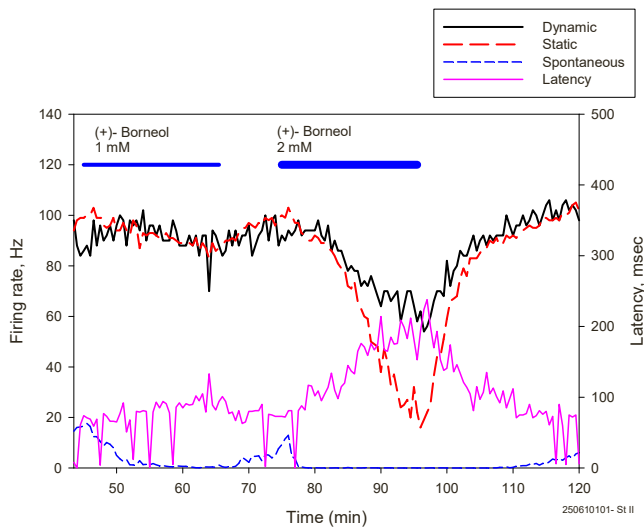


Fig. 4. The effects of low (1 mM) and high (2 mM) concentrations of borneol, applied for 20 min to an St II unit. The lower dose had a marginal effect on the dynamic and static phase response components and response latency. The spontaneous firing was almost silenced. After recovery the higher dose reduced all the activities to a greater extent, and the response latency increased. Recovery was obtained 20 min post application. Explanation for the axes and key are given in the Fig. 1 legend.

Table 1

Summary of the effects of compounds tested. Application of one-sample *t* tests: * *p* < .05 and ** *p* < .01.

Compound	N	Group mean % change			Latency change (ms)	Dose
		Spontaneous	Dynamic	Static		
(+)-Borneol	9	-74 *	2	-21	13	19.4
(+)-Camphor	28	-97 **	-23 **	-35 **	96 **	18.5
(-)-Camphor	11	-89 *	-24 *	-25 *	185 *	18.3
(+)-Carveol	3	-99	-84	-99 *	200 *	18.6
Eugenol	4	-100	-100 *	-99 **	738	13.0
Thymol	15	-81	-76 **	-79 **	276	16.0
Lignocaine	7	-33.3	-70 **	-83.5 **	108.3	8.0

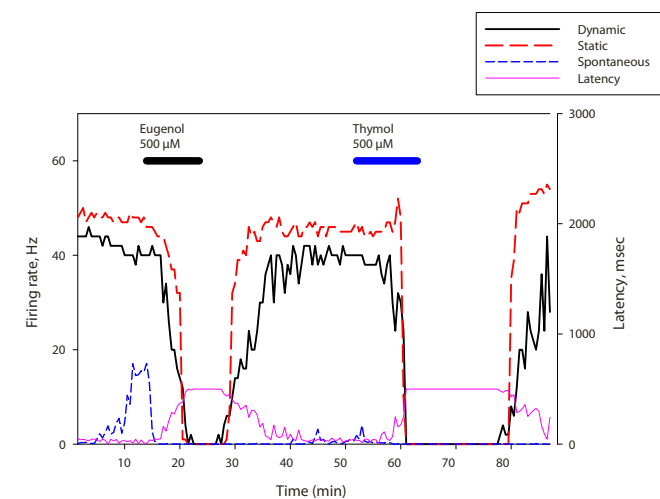


Fig. 5. The effects of applications of eugenol and thymol, both given at 500 µM, on the firing activity of an St II unit. Thymol was more effective than eugenol at depressing the unit at the same dose (500 µM for 20 min). Explanation for the axes and key are given in the Fig. 1 legend.

St II units for all kinds of activity, though static and latency difference reached statistical significance (*p* = .030 and .029 respectively, *N* = 11). The same was true for (+)-camphor but failed to reach statistical significance (despite much larger sample size) except for the latency change (*p* = .025, *N* = 28). Due to the small sample sizes for other compounds divided by unit type, it made little sense to investigate their differential effect on St I and St II units.

Some experiments looked at the effects of the local anaesthetic lignocaine. The results from one such experiment is shown in Fig. 6.

There was an indication that the depressant effects of both camphor and lignocaine could be reversed with 3 mM TEA, see Fig. 7b & c. The reversing effects reached statistical significance for camphor, using a 2-way repeated measures ANOVA, during drug versus + blocker, *F*(1,5) = 8.04, *p* = .036, partial η^2 = 0.62. In 4 St II units the possible TRP channel blocking effects of ruthenium red were tested on depressant effects of camphor. No blocking effect of camphor effects was seen, see Fig. 7a. No obvious differences between St I and St II units were observed in any of these blocking/reversal experiments.

Discussion

The camphor and camphor-related monoterpenoid compounds tested in these experiments, in the range 0.5–2 mM concentration, had dose-dependent depressant effects on both mechanically evoked (dynamic and static phases) and spontaneous firing of slowly adapting mechanoreceptors (Figs. 2–4). Concurrently the latency of the first spike of the response to the mechanical ramp was increased by the compounds, see Table 1 and Figs. 1–6. Both types of mechanoreceptor, St I and St II, were similarly affected, although (-)-camphor had a greater depressant effect on St II than on St I units. Otherwise, the effects of the two camphor enantiomers were very similar. There are few published studies that have examined the different pharmacological effects of the camphor enantiomers. One study found that the two enantiomers had equal positive modulation of recombinant human GABA_A receptor EC₂₀ currents (Hall et al., 2004). The results with camphor confirm the depressant effects previously described in St units using a similar dose range (Cahusac, 2009). Statistically significant differences were found across the different compounds for their effect on the dynamic and static phases. Thymol was found to have the greatest depressant effect on these phases. Eugenol appeared to have the greatest depressant effects and was most potent (see Table 1, except for direct comparison shown in

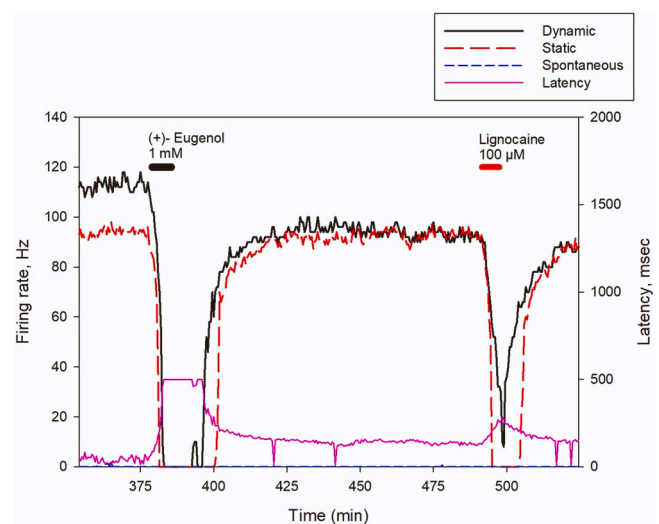


Fig. 6. In an St II unit, the depressant effect of local anaesthetic lignocaine at 100 µM appears qualitatively similar to the preceding application of (+)-eugenol 1 mM, both applied for 6 min. Explanation for the axes and key are given in the Fig. 1 legend.

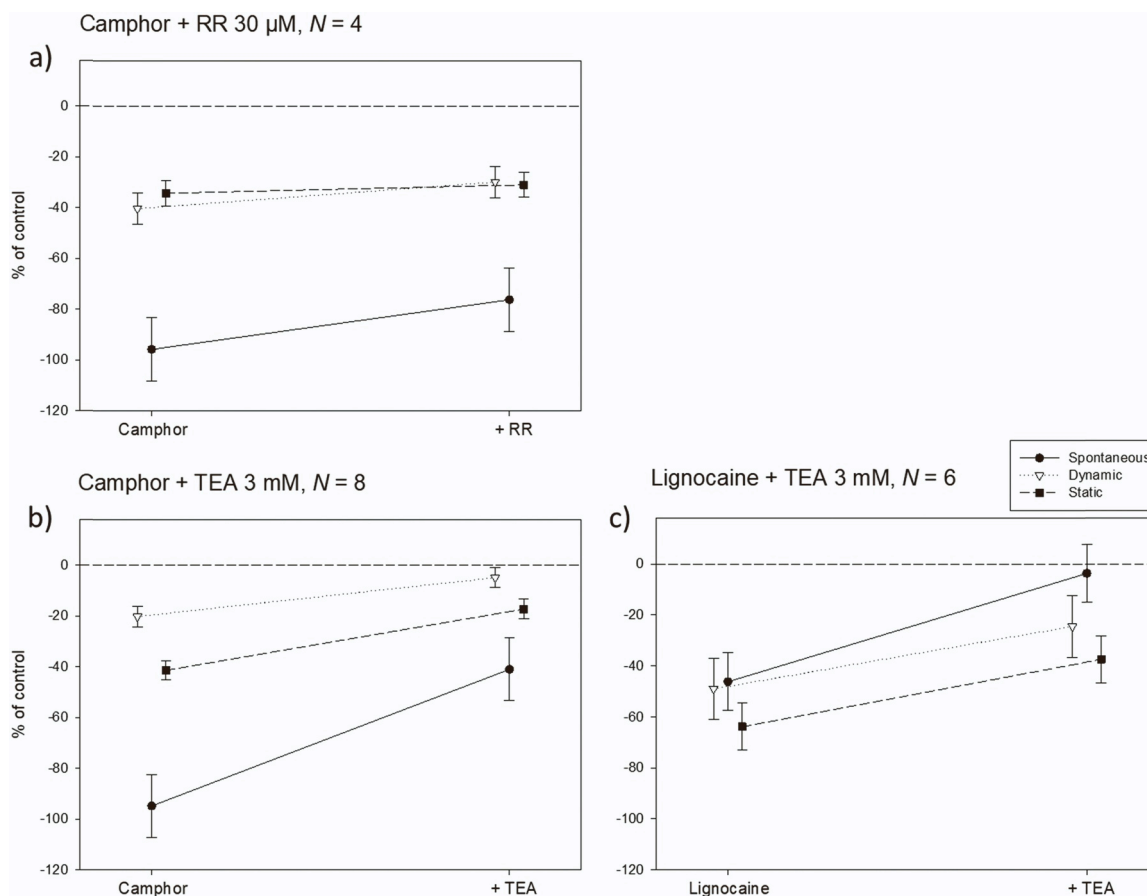


Fig. 7. Effects of ruthenium red (RR) and TEA on camphor and lignocaine depressions of neural activity. In each experiment the depressant drug was applied for 10 min after which RR or TEA were added to the bath for a further 10 min. a) shows that the depressant effect of camphor was unaffected by RR. b) shows that the depressant effects of camphor could be reversed by TEA ($p = .036$). c) the depressant effects of lignocaine appear to be reversed by TEA. In each plot the phases for the types of neural activity are indicated by the key, so that filled circle: spontaneous; inverted triangle: dynamic; filled square: static. All points represent the mean % change in spike counts relative to before the depressant drug (camphor or lignocaine) were applied (horizontal dashed line represents activity prior to depressant drug). The error bars represent standard errors of the mean, calculated for within-participant effects (Loftus and Masson, 1994).

Fig. 5), although too few experiments were done to allow inclusion of it or carveol in the statistical analyses. It is notable that thymol and eugenol are monocyclic monoterpenes, as distinct from camphor and borneol which are bicyclic monoterpenes (Zielińska-Błajet and Feder-Kubis, 2020), so their relative efficacy may depend on their cyclic structure.

Experiments using the broad spectrum TRP blocker ruthenium red, failed to obtain evidence that the depressant effects of camphor could be reversed. This finding is consistent with work using calcium micro-fluorimetry that revealed non-adapting cold-sensitive dorsal root ganglion cells displaying camphor sensitivity despite the presence of ruthenium red (Babes et al., 2006). The depressant effects of the compounds appeared similar to those obtained using the local anaesthetic lignocaine. The depressant effects of camphor and lignocaine were similarly reversed by the K^+ channel blocker TEA. This suggests that part of the action of these compounds may involve enhancement of K^+ channels.

It is tempting to believe that TRP channels are involved in the actions of the monoterpenoid compounds examined in the present study. Camphor at 1 mM had no action at the TRPM8 cold channel (McKemy et al., 2002). Using concentrations of 1 – 10 mM, (-)-camphor and (+)-camphor enantiomers activate and desensitise the capsaicin receptor (TRPV1), although the currents were smaller than those produced by 1 μ M capsaicin (Xu et al., 2005). The same study found that camphor inhibited the TRPA1 channel. A previous study found that mustard oil (100 μ M) and capsaicin (1 – 10 μ M) had no effect, suggesting that

TRPV1 and TRPA1 channels are not active in slowly adapting mechanoreceptors (Cahusac, 2009). High concentrations (10 mM) of camphor activated TRPV1 and TRPV3 channels (Nguyen et al., 2021). The current study found depressant effects by compounds used at lower concentrations, between 0.5 and 2 mM. Previously it was found that a number of TRP channel agonists (including (+)-camphor, menthol, cinnamaldehyde) had depressant effects on St units (Cahusac, 2009). It is interesting that these compounds inhibit basal phospholipid C (PLC) activity in HEK293T cells (Kim et al., 2008). Together with the lack of effects of ruthenium red (broad spectrum TRP channel blocker (Zhang et al., 2012)) makes it unclear whether they are acting at TRP channels. Our data are also consistent with a non-specific effect similar to the action of local anaesthetic. Perhaps further research is required using more selective antagonists, for example using AMG8432 for the TRPV3 channel (Ishikawa et al., 2019).

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Conflict of Interest Statement

The authors have no conflict of interest of any kind in relation to this paper.

Data Availability

The data will be openly available as Supplementary information.

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We are grateful to Glasgow Caledonian University, Department of Pharmacology for financial support and technical support. We are also grateful to the Animal Facility support at the Psychology Department of Stirling University.

Statement of Ethics

The Ethics Committee of the Stirling University Department of Psychology approved the use of animals in this study. All experimental procedures complied with UK Home Office regulations as well as the European Directive 86/609/EEC.

Author Contributions

The ideas for the work were from Cahusac who also set up the preparation, ran experiments, analysed the data and wrote the paper. Veermalla aided in setting up and running the experiments, and approved the final manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ibneur.2022.07.002](https://doi.org/10.1016/j.ibneur.2022.07.002).

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