

Eucalyptus, Lemon, Peppermint,
and Rock Rose and Stress in
College Students:

How to Use Aromatherapy Case
Studies in Undergraduate
Education to Teach Integrated
Chemistry and Biology

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Presentation Description

- A group of 4 undergraduate research students wanted to build a unified study to explore **how essential oils would impact college student stress**.
- **Over the course of an academic year**, each student developed a detailed understanding surrounding one plant and its essential oil.
- Together they **built a unified, double-blind experiment** that worked to test established primary literature findings in aromatherapy on a unique population.
- **FINDINGS**: These research students needed to develop key concepts in biology, chemistry, botany, psychology, and aromatherapy to execute the experience well. In addition, they cross-analyzed their data to test their independent and collective hypotheses. **The overall experience provided an opportunity for deeper student learning and a stronger appreciation of cultural botanical uses while expanding the knowledge in aromatherapy.**



Educational Idea: Use EOs to Train Students in Biology and Chemistry

- 4 students
 - 2 seniors
 - 2 juniors
 - 2 Graduating with Honors in their Major
 - 1 Honors Program student
 - 2 Senior Thesis students

What the Students Designed

A double-blind study to examine essential oil impacts on college students

Inhaler Development



- **Materials**

- Individual nasal inhalers
- Four essential oils:
 - Peppermint (*Mentha piperita*)
 - Lemon (*Citrus limon*)
 - Cistus (*Cistus ladanifer*)
 - Eucalyptus (*Eucalyptus globulus*)
- Extra virgin olive oil for the control inhaler

- **Preparation**

- 16 inhalers of each type (total of 80)
- 8 drops of respective oil administered to the cotton wick

- **Safety and Integrity**

Experiment Methodology

- Use surveys to gain information:
 - Demographic
 - Baseline
 - Check-In
 - Post-Dose
 - Exit Study
- Measure stress and mood levels through PSS and WEMWBS in both baseline and post-dose surveys
- Self-administer with cotton wick inhalers, then take post-dose survey (Google form)
- Statistical data analysis



Psychology Tests Used by the Student Research Group

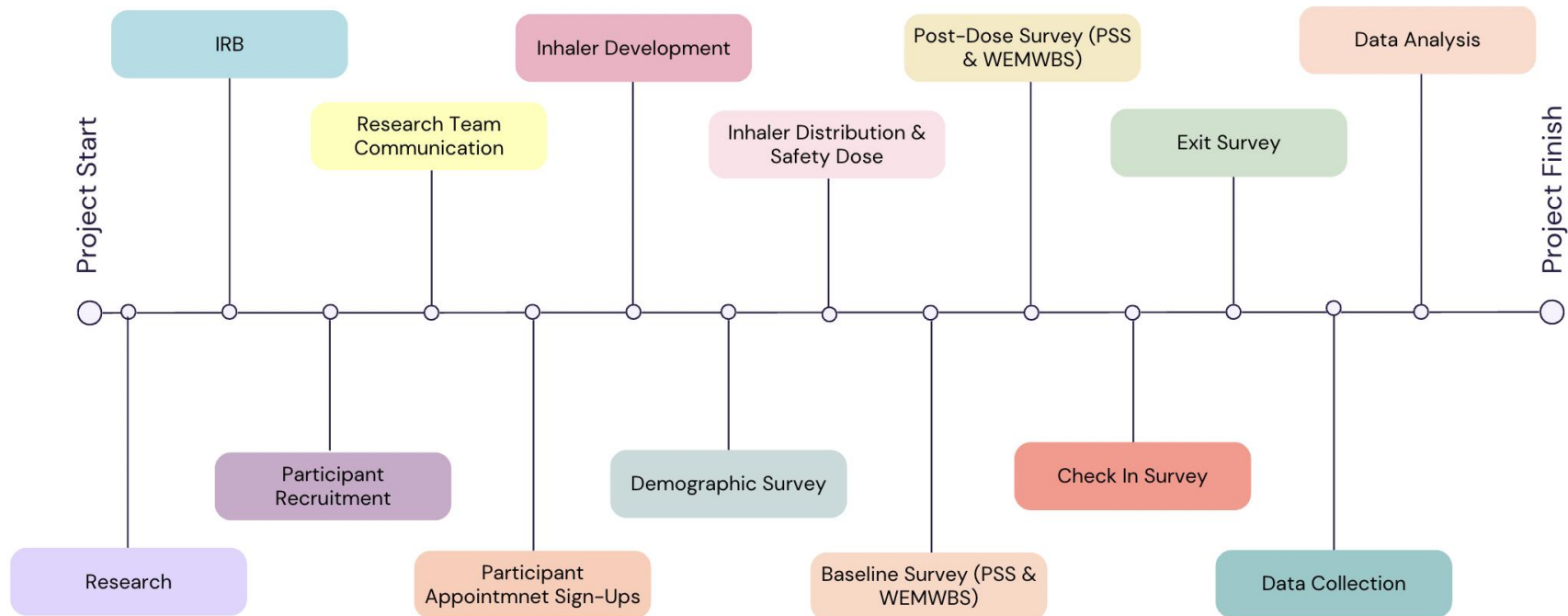
STATEMENTS	None of the time	Rarely	Some of the time	Often	All of the time
I've been feeling optimistic about the future	1	2	3	4	5
I've been feeling useful	1	2	3	4	5
I've been feeling relaxed	1	2	3	4	5
I've been feeling interested in other people	1	2	3	4	5
I've had energy to spare	1	2	3	4	5
I've been dealing with problems well	1	2	3	4	5
I've been thinking clearly	1	2	3	4	5
I've been feeling good about myself	1	2	3	4	5
I've been feeling close to other people	1	2	3	4	5
I've been feeling confident	1	2	3	4	5
I've been able to make up my own mind about things	1	2	3	4	5
I've been feeling loved	1	2	3	4	5
I've been interested in new things	1	2	3	4	5
I've been feeling cheerful	1	2	3	4	5

**For each question choose from the following alternatives:
0 - never 1 - almost never 2 - sometimes 3 - fairly often 4 - very often**

- _____ 1. In the last month, how often have you been upset because of something that happened unexpectedly?
- _____ 2. In the last month, how often have you felt that you were unable to control the important things in your life?
- _____ 3. In the last month, how often have you felt nervous and stressed?
- _____ 4. In the last month, how often have you felt confident about your ability to handle your personal problems?
- _____ 5. In the last month, how often have you felt that things were going your way?
- _____ 6. In the last month, how often have you found that you could not cope with all the things that you had to do?
- _____ 7. In the last month, how often have you been able to control irritations in your life?
- _____ 8. In the last month, how often have you felt that you were on top of things?
- _____ 9. In the last month, how often have you been angered because of things that happened that were outside of your control?
- _____ 10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

Project Timeline of Key Research Events

Year-long study for research students



33-day trial for participants

Essential Oil Findings



- No significant data was found for any test that were run
- No difference between all the treatments (EOs and control)

Participant Issues

Compliance of the total participant population was low

Compliance declined over the study period

Could be due to losing inhalers, forgetting to document dosage or dosing at all

Participants may not have felt stressed (Baseline WEMWBS = most likely not depressed, Baseline PSS= moderate stress)

Participants may have been given unclear instructions, or the surveys may have been hard to complete

Additionally, oils may have had an unfavorable smell

Conclusions After Examining Survey Responses

Most participants did feel EOs were useful and helpful in some aspect

Participants felt comfortable and enjoyed being in the aromatherapy study

No significant findings, but this opens the door for further investigations into human aromatherapy research on mental health in young populations

Negative findings are an integral part of scientific progress

Future Direction

RUN THE SAME EXPERIMENT
MAKE DOSING AND SURVEYS
MANDATORY EVERY DAY

SURVEY LOCATED IN AN EASILY
ACCESSIBLE PLACE (EX.
WEBSITE)

COME INTO THE LAB AND
PERFORM AN INDUCED STRESS
TEST

IF PARAMETERS COULD NOT
CHANGE, MORE ADVERTISING
COULD BE DONE AND
HOLDING A LONGER
RECRUITMENT PERIOD GOAL
NUMBER OF PARTICIPANTS
WOULD BE 100-150



What the Students Learned!

Examples from student work

Origins and Early Uses

- *Mentha* species have a long history, originating in the Mediterranean region.
- Cultivated for centuries for medicinal and culinary purposes (Mansori, 2014).
- Archaeological evidence shows ancient Egyptians, Greeks, and Romans used mint for healing and flavoring.

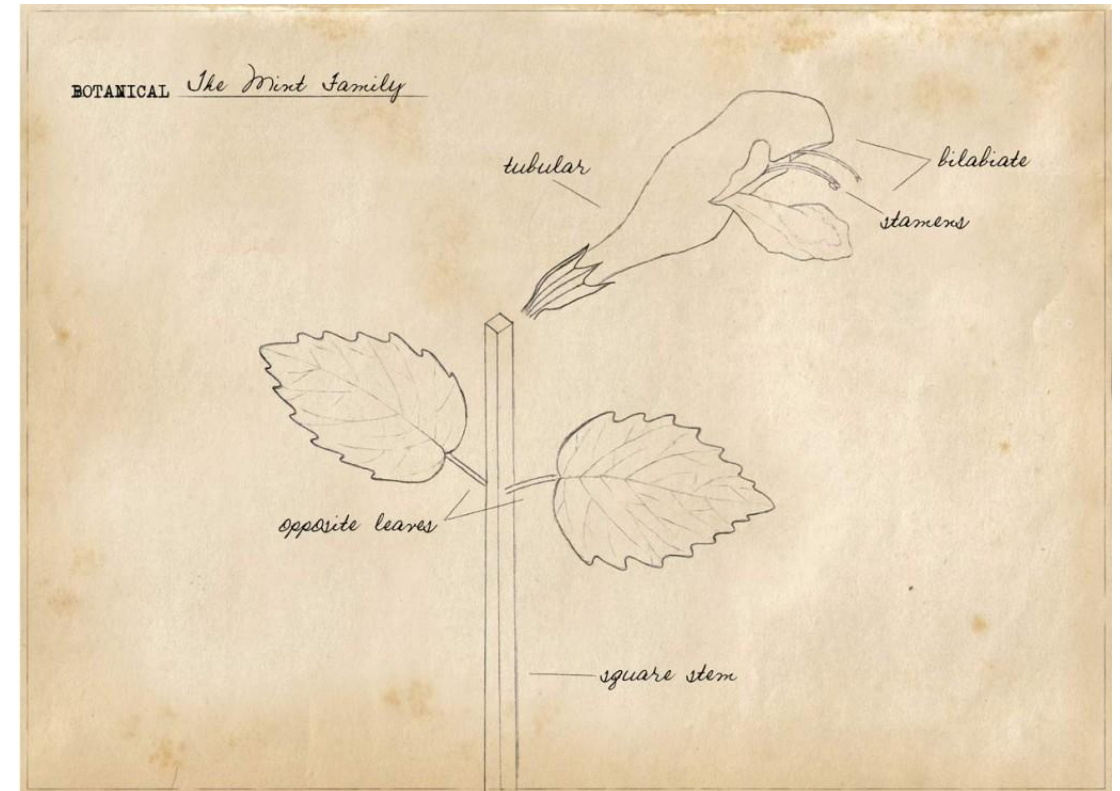


Botanical Characteristics

- The Lamiaceae family (mint family) has distinctive features.

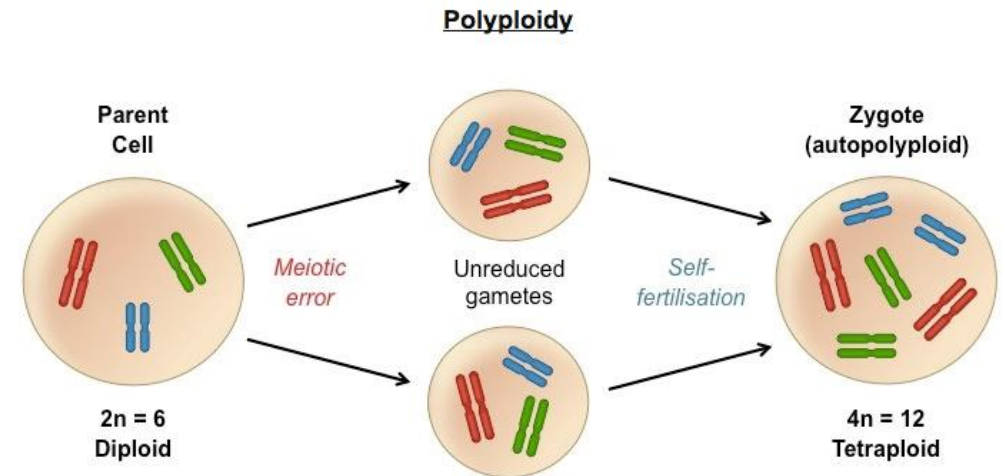
Key Characteristics:

- Square stems
- Opposite leaf arrangement
- Tubular flowers
- Bilabiate (two-lipped) corolla - crucial for attracting pollinators.



Genetic Complexity

- Significant genetic complexity within *Mentha*.
- **Key Factors**
- **Hybridization:** Interbreeding of genetically distinct individuals.
 - A substantial portion of the genus comprises hybrids (López-Caamal et al., 2014).
 - Complicates clear classification.
- **Polyploidy:** Presence of more than two complete sets of chromosomes.
 - Common among *Mentha* hybrids (Gobert et al., 2022).
 - Plants possess multiple copies of their entire genome.

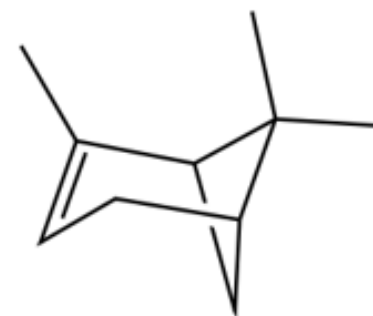


Example of Chemical Understanding

α -Pinene (37.36% composition) ($C_{10}H_{16}$) is a natural terpenoid hydrocarbon commonly found in coniferous trees among other sources. Studies show that α -Pinene has useful medicinal properties such as altering antibiotic resistance, anticoagulation, antitumor, antimicrobial, antioxidant, and anti-inflammatory effects. Additionally, α -Pinene suppresses the production of inflammatory molecules such as IL-6 and TNF- α and the activity of MAPKs and NF- κ B (18).

Figure 4. α -Pinene. [Scifinder](#). *American Chemical Society*.

[" \$\alpha\$ -Pinene" All Search | CAS SciFinder](#)



Connections to Methodology: GC-MS

MONOTERPENE	
limonene (d)	64.17%
β -pinene	11.00%
γ -terpinene	10.56%
α -pinene	1.99%
sabinene	1.99%
β -myrcene	1.76%
α -thujene	0.49%
terpinolene	0.45%
p-cymene	0.41%
α -terpinene	0.23%
(E)- β -ocimene	0.14%
α -phellandrene	0.06%
(Z)- β -ocimene	0.06%
camphene	0.05%

MONOTERPENOL	
α -terpineol	0.14%
linalool	0.11%
geraniol	0.04%
terpinen-4-ol	0.03%
nerol	0.03%
citronellol	0.02%

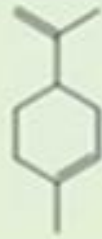
SESQUITERPENE	
β -bisabolene	0.72%
(E)- α -bergamotene	0.53%
β -caryophyllene	0.27%
(Z)- α -bergamotene	0.04%
bicyclogermacrene	0.04%

ESTER	
neryl acetate	0.62%
geranyl acetate	0.39%

ALDEHYDE	
geranial	1.84%
neral	1.12%
citronellal	0.13%
decanal	0.04%

Limonene (15-98 %)

- Effective in Alzheimer's disease, insomnia, dementia, migraine, anxiety and stress related disorders



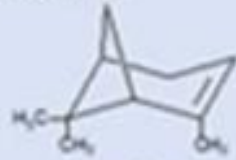
1,8- Cineole (up to 26 %)

- Enhances cytokines along with oxidative stress and NF- κ B activity
- Mucolytic and spasmolytic action on the respiratory tract, relieve anxiety

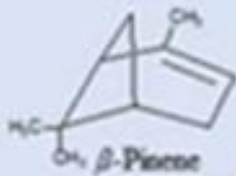


α - Pinene (0.30 – 0.98 %) and β - Pinene (0.80 – 25.44 %)

- Anxiolytic-like effects
- Influence multiple neurotransmitter, inflammatory and neurotrophic signals as well as behaviour, demonstrating psycho-activity
- Effective in stroke, ischemia, inflammatory and neuropathic pain (migraine), cognitive impairment (relevant to Alzheimer's disease and ageing), insomnia, anxiety, and depression



α -pinene

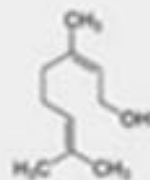


β -Pinene



Thymol (up to 10 %)

- Helps relieve respiratory congestion and inflammatory conditions; anti-inflammatory, antiviral, antibacterial, and antiseptic (mainly in the treatment of the upper respiratory system)



7-Terpinene

Bioactive Chemicals in Essential Oils

8-Cymene

- Bioactive compounds found in citrus essential oil and their therapeutic effects (Agarwal et al., 2022)

9-Limonene

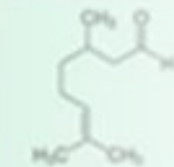
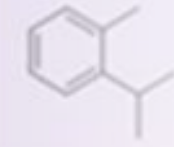
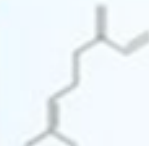
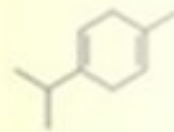
- Anxiolytic-like effects

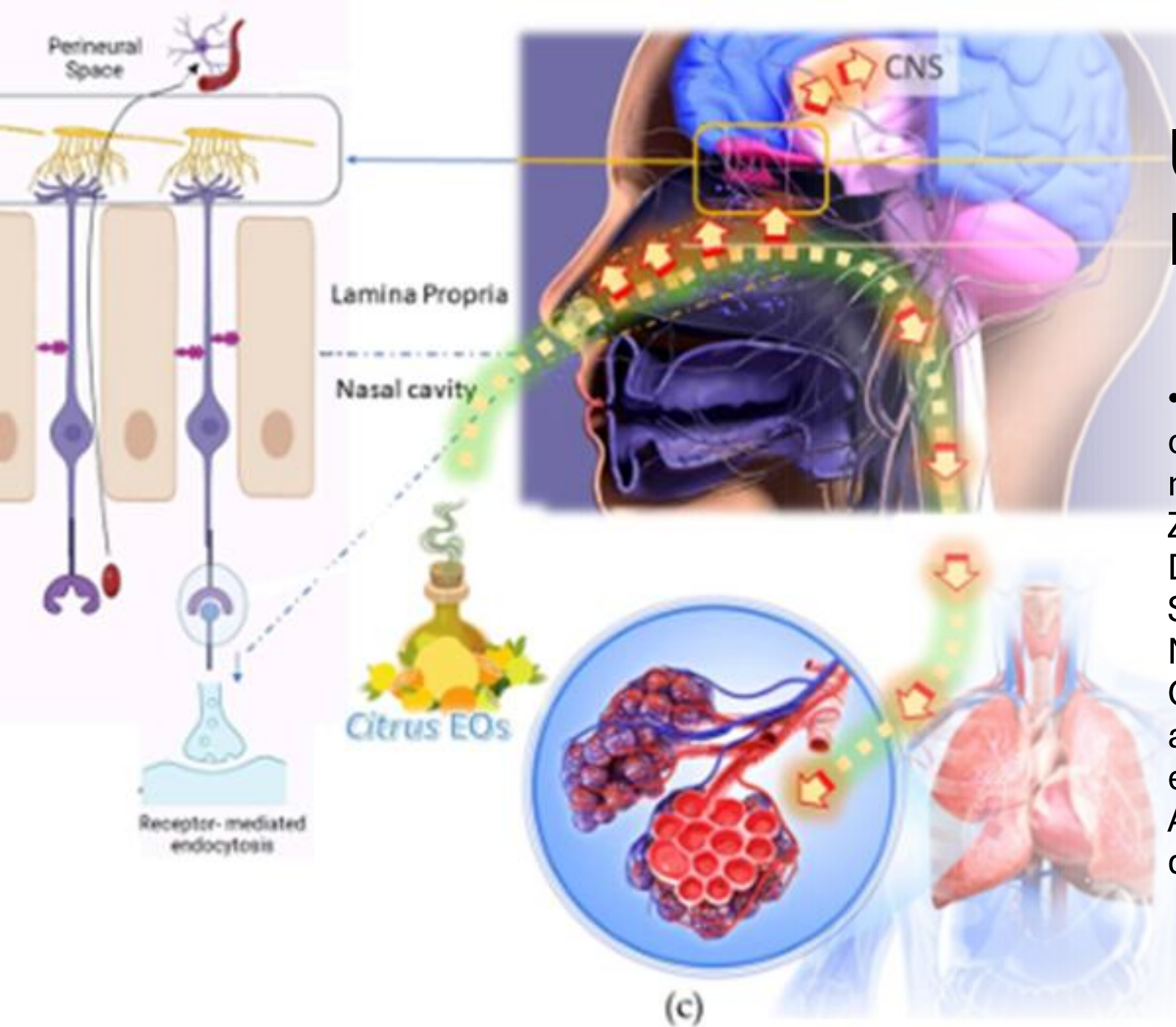
Citronellal

- Uplifts mood
- Slows or prevents growth of bacteria, fungi

Camphene

- Potent antimicrobial
- Anti-congestive respiratory effects, treating cardiovascular lipoproteins



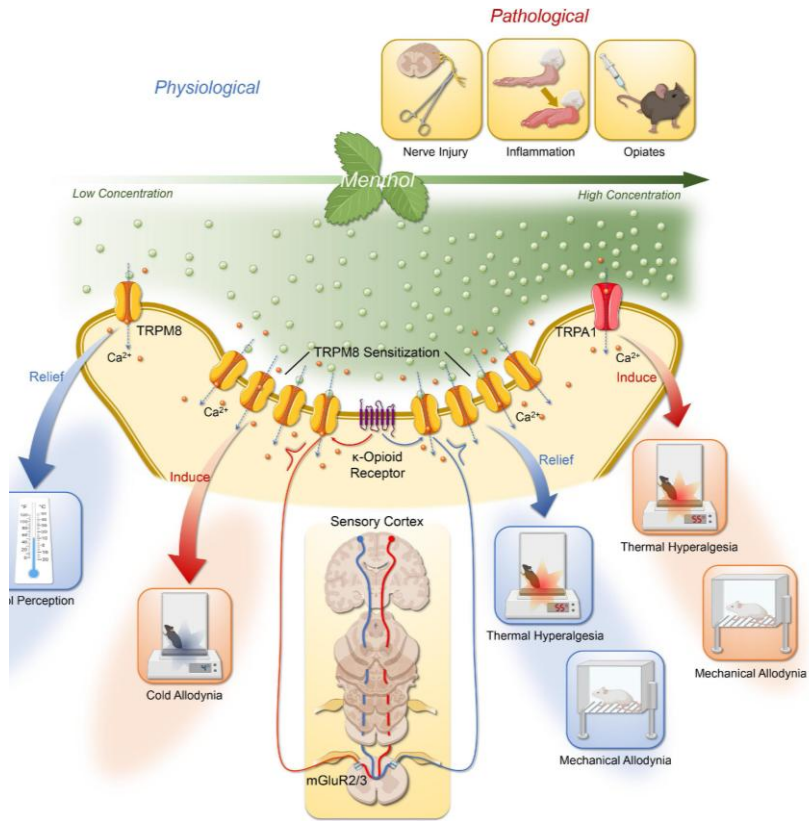


Understanding of Inhalation

- Inhalation of citrus essential oils pathway of delivery from nasal cavity to CNS. Agarwal, P., Z. Sebghatollahi, M. Kamal, A. Dhyani, A. Shrivastava, K.K. Singh, M. Sinha, N. Mahato, A.K. Mishra, and K.-H. Baek. 2022. Citrus essential oils in aromatherapy: therapeutic effects and mechanisms. *Antioxidants*. 11:2374. doi:10.3390/antiox11122374.

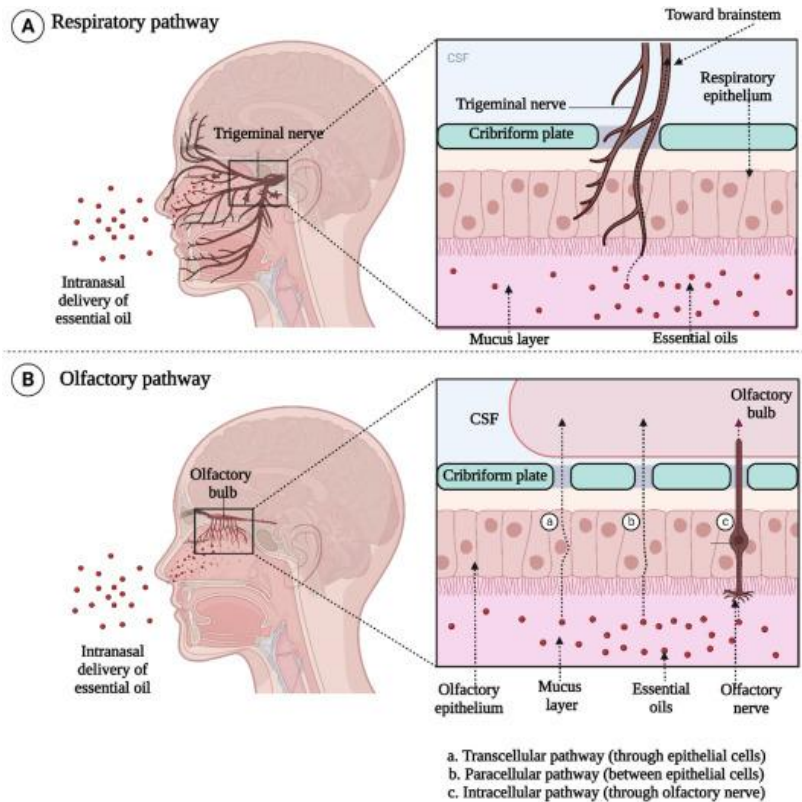
(c)

Analgesic Therapeutic Process



- **Activation of the TRPM8 Receptor**
- **Peripheral Action**
- **Cooling Sensation as Counterirritant**
- **Gate Control Theory**

Stress Reduction Therapeutic Properties



Respiratory Pathway

- Intranasal Delivery
- Interaction with Trigeminal Nerve
- Essential Oil Interaction
- Signal Transmission

Olfactory Pathway

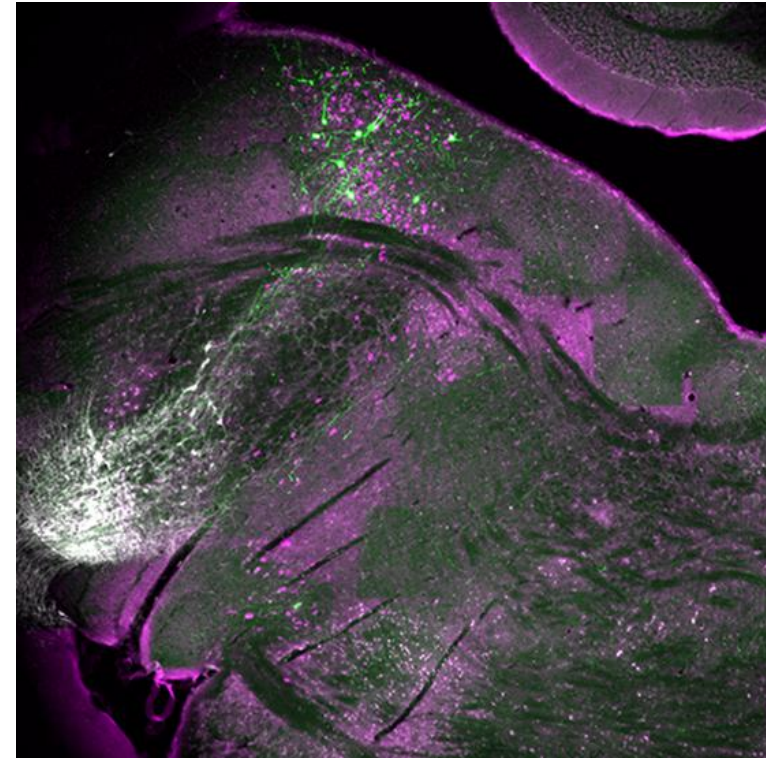
- Intranasal Delivery
- Interaction with Olfactory Epithelium
- Olfactory Receptors
- Signal Transmission to Olfactory Bulb
- Limbic System and Stress

Going Even Deeper: Effects of Lemon on the Brain on Mood and Stress

D-limonene has antidepressant effect on the brain of mice, affecting production of serotonin and dopamine

Mood-elevating properties in mice through dopaminergic system using 5-HT_{1A} receptors

stress-relieving properties in mice through inhibitory effects on NE, 5-HT, and DA turnover by R-limonene and S-limonene



Drd2-Pet1 neurons found in dorsal raphe of mouse brain (green). Serotonin and dopamine shown in mouse brain (purple, white) (Lyon et al., 2020)

Pedagogical Conclusions



Essential oils provide an excellent source to train students in an integrated STEM example



Essential oils capture the imagination of undergraduate students and hold their attention to research



Working in a semi-clinical setting helps students make choices about their career choices between academic or professional school

Thank You for Listening!

Questions?

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