Effects of vapor exposure to Δ9-tetrahydrocannabinol (THC) in the Maine Lobster (*Homarus americanus*)

Arnold Gutierrez[1], Kevin M. Creehan[1], Mitchell Turner[1,3], Rachel Tran[1,4], Tony M. Kerr[2], Jacques D. Nguyen[1,2], and Michael A. Taffe[1,2]

[1]*Department of Psychiatry, University of California, San Diego; La Jolla, CA*
[2]*Department of Neuroscience, The Scripps Research Institute; La Jolla, CA*
[3]*Colorado College; Colorado Springs, CO*
[4]*University of Washington; Seattle WA*

Abstract

Rationale: Despite a long history of use in synaptic physiology, the lobster has been a neglected model for behavioral pharmacology. A restauranteur proposed that exposing lobster to cannabis smoke reduces anxiety and pain during the cooking process. It is unknown if lobster gill respiration in air would result in significant Δ9-tetrahydrocannabinol (THC) uptake and whether this would have any detectable behavioral effects.

Objective: The primary goal was to determine tissue THC levels in the lobster after exposure to THC vapor. Secondary goals were to determine if THC vapor altered locomotor behavior or nociception.

Methods: Tissue samples were collected from muscle, brain and hemolymph of Homarus americanus (N=3 per group) following 30 or 60 minutes of exposure to vapor generated by an e-cigarette device using THC (100 mg/mL in a propylene glycol vehicle). Separate experiments assessed locomotor behavior and hot water nociceptive responses following THC vapor exposure.

Results: THC vapor produced duration-related THC levels in all tissues examined. Locomotor activity was decreased (distance, speed, time-mobile) by 30 min inhalation of THC. Lobsters exhibit a temperature-dependent withdrawal response to immersion of tail, antennae or claws in warm water; this is novel evidence of thermal nociception for this species. THC exposure for 60 minutes had only marginal effect on nociception under the conditions assessed.

Conclusions: Vapor exposure of lobsters, using an e-cigarette based model, produces dose-dependent THC levels in all tissues and reduces locomotor activity. Hot water nociception is temperature dependent in the lobster, but no clear effects of THC inhalation were confirmed.

Keywords: crustacean; e-cigarette; nociception; cannabis

1. Introduction:

In the early fall of 2018, a minor media storm described a seafood restaurant in Maine (USA) that was proposing to expose lobsters to marijuana smoke prior to cooking (Stone, 2018). At least three testable assertions were made including that some psychoactive constituent of cannabis would be transferred to the lobster via open air respiration (see follow-up reporting; (Grunewald, 2019), that this would have specific behavioral effects similar to those produced in vertebrates and that the cooking process would remove intoxicating psychoactive constituents from the meat thereby rendering it safe for human consumption. This latter assertion was related to a claim that "a steam as well as a heat process" would bring the lobster to 420 °F (Hinckley, 2018), which would presumably require broiling or oven baking in preference to the more typical boiling or steaming cooking method. These assertions lead

Address Correspondence to: Dr. Michael A. Taffe, Department of Psychiatry, Mail Code 0714; 9500 Gilman Drive; University of California, San Diego, La Jolla, CA 92093; USA; Email: mtaffe@ucsd.edu

to at least two key questions. Can air exposure to Δ9-tetrahydrocannabinol (THC), the primary psychoactive constituent of cannabis, produce significant tissue levels of the drug in lobsters? If so, does it have any discernible behavioral effects?

Lobsters are aquatic species that respirate via gills located inside their carapace. Lobsters can survive in air for many hours up to a few days, as they are able to keep their gills wet enough to function, but they do go into oxygen debt, e.g. across a 24 h emersion from water (Couillard and Burridge, 2015; Forgan et al., 2014). It is unclear if the gill structures would support the uptake of THC that is rendered airborne via smoke particulate or Electronic Drug Delivery System (EDDS or e-cigarette) device vapor. We recently demonstrated that vapor inhalation of THC using an e-cigarette based approach produces anti-nociceptive effects and reduces the body temperature and spontaneous activity of male and female rats (Javadi-Paydar et al., 2018; Nguyen et al., 2016). These are canonical effects that are observed after injection or oral delivery of THC in laboratory vertebrates including in rats (Taffe et al., 2015; Thompson et al., 1973), mice (Beardsley et al., 1987), monkeys (McMahon and Koek, 2007; Taffe, 2012) and dogs (Fitzgerald et al., 2013; Thompson et al., 1973), although the individual behavioral or physiological outcomes may be observed after different doses. Behavioral and physiological effects, and plasma THC levels, of THC delivered by vapor inhalation depend on the exposure duration as well as the dose administered. We have shown that dose can be controlled during inhalation exposure by varying either the concentration of THC in the e-liquid vehicle or the duration of exposure at a fixed concentration (Nguyen et al., 2016; Taffe et al., 2021). This validated platform is therefore ideal to test the hypothesis that aerosol THC exposure of lobsters has physiological effect.

Traditional cooking of lobster is by immersion of live animals in either boiling water or steam leading to concerns by some that the animal might experience pain. Indeed, live cooking has been banned in Switzerland. There is no available evidence demonstrating clearly that lobsters are sensitive to temperature, however one paper has shown that crayfish respond to a hot metal rod stimulus applied to the claw (Puri and Faulkes, 2015). It is thus of interest to develop assays to determine if lobsters exhibit thermal nociceptive behavioral responses and then to determine if those responses can be altered by THC exposure. The

hot-water tail withdrawal assay in rats involves a reflexive tail movement when it is inserted in hot water (~48-52°C) and has been shown to be altered in rats after vapor inhalation of THC (Javadi-Paydar et al., 2018; Nguyen et al., 2016; Nguyen et al., 2020). Thus, one goal was to determine if a warm water immersion test of nociception is functional in the lobster and if so, if THC exposure decreased thermal nociception as it does in rodents (Tseng and Craft, 2001; Wiley et al., 2007). As part of a model development, it was important to determine if different responses could be obtained from tail, claw or antenna immersion and if the response depended on the temperature of the water bath, as in (Javadi-Paydar et al., 2018).

Development of different animal model species, including invertebrates, for the evaluation of drug effects can offer both unique and converging advantages, as recently reviewed (Smith, 2020). The lobster is an established model for evaluating neuronal morphology, central pattern generation and synaptic mechanisms in the stomatogastric ganglion (Eisen and Marder, 1984; Marder and Eisen, 1984; Thirumalai and Marder, 2002). The lobster can be studied within institutions that are not equipped to oversee vertebrate animal research, or can be studied at reduced expense in institutions where vertebrate research is supported. A recent review indicates there are no clear data on lobster nociception and arthropod investigations of nociception do not typically involve thermal stimuli (Walters, 2018) and only one available report shows that crayfish are sensitive to a thermal stimulus delivered by soldering iron (Puri and Faulkes, 2015).Thus it serves the additional goal of determining if thermal nociception exists in this crustacean species.

There is very limited evidence on whether the lobster would be sensitive to THC exposure, however, the neuromuscular junction of lobsters appears to be regulated, in part, by cannabinoid mechanisms. Turkanis and Karler (1988) showed that THC had dose-related effects on excitatory neuromuscular junction potential amplitudes, increasing them at moderate concentrations and decreasing amplitude at higher concentrations (Turkanis and Karler, 1988). This enhances confidence that some endocannabinoid mechanisms are present in the lobster and that THC might affect locomotor behavior. Hypolocomotion is a canonical sign of cannabinoid action in rats (Tseng and Craft, 2001; Wiley et al., 2007) and mice (Wiley, 2003), and occurs after vapor inhalation of THC

2

(Javadi-Paydar et al., 2018; Taffe et al., 2021). Thus, locomotor activity was selected to assay for evidence of *in vivo* behavioral effect. Less directly, recent studies in the crayfish, a related aquatic crustacean, have shown locomotor effects of cocaine, morphine and methamphetamine (Imeh-Nathaniel et al., 2017) and intravenous self-administration of amphetamine (Huber et al., 2018; Huber et al., 2011). This further suggests that behavioral pharmacological effects of recreational drugs can be effectively assessed in the lobster.

2. Methods:

2.1 Subjects

Wild caught female and male Maine lobster (*Homarus americanus;* ~0.7-0.9 kg) were obtained from a local supermarket. When housed longer than several hours in the laboratory, the animals were maintained in chilled (~6-10 °C), aerated aquariums (2-3 per 20 gallon tank) and fed with frozen krill, fish flakes and anacharis. The pharmacokinetic studies were conducted under protocols approved by the Institutional Animal Care and Use Committee (IACUC) of The Scripps Research Institute due to a decision that a protocol was required for this invertebrate species. The remaining studies were conducted at the University of California, San Diego where the institution does not require protocol supervision / approval for this invertebrate species.

2.2 Inhalation Apparatus

Sealed exposure chambers were modified from the 259mm X 234mm X 209mm Allentown, Inc (Allentown, NJ) rat cage to regulate airflow and the delivery of vaporized drug to the chamber using e-cigarette cartridges (Protank 3 Atomizer, MT32 coil operating at 2.2 ohms, by Kanger Tech; Shenzhen Kanger Technology Co.,LTD; Fuyong Town, Shenzhen, China; controlled by e-vape controller Model SSV-1; La Jolla Alcohol Research, Inc, La Jolla, CA, USA) or (SMOK® TFV8 with X-baby M2 atomizer; 0.25 ohms dual coil; Shenzhen IVPS Technology Co., LTD; Shenzhen, China; controlled by e-vape controller Model SSV-3; La Jolla Alcohol Research, Inc, La Jolla, CA, USA) as has been previously described(Javadi-Paydar et al., 2019; Javadi-Paydar et al., 2018; Nguyen et al., 2016). The controllers were triggered to deliver the scheduled series of puffs by a computerized controller designed by the equipment manufacturer

(La Jolla Alcohol Research, Inc, La Jolla, CA, USA). The chamber air was vacuum controlled by a chamber exhaust valve (i.e., a "pull" system) to flow room ambient air through an intake valve at ~1 L per minute. This also functioned to ensure that vapor entered the chamber on each device triggering event. The vapor stream was integrated with the ambient air stream once triggered. The chambers were empty of any water or bedding material for these exposures.
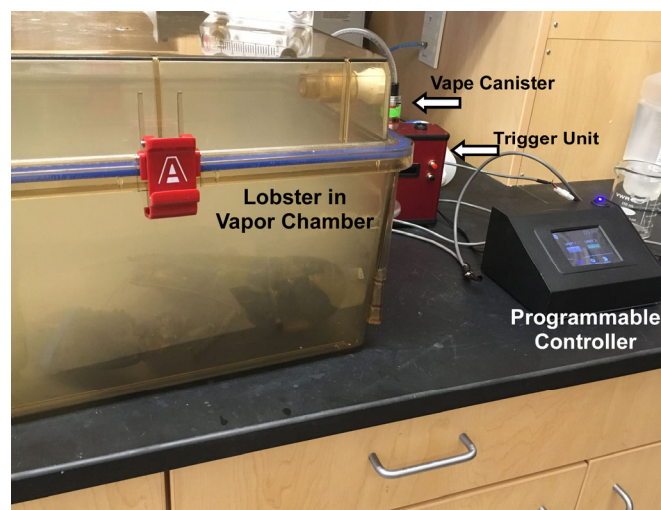
Figure 1: *Depiction of a lobster undergoing exposure to THC vapor in a sealed chamber. Components of the vapor delivery system are identified.*

2.3 Drugs:

Lobsters were exposed to vapor generated from Δ^9-tetrahydrocannabinol (THC; 100 mg/mL) dissolved in a propylene glycol (PG) vehicle. For the Protank3 based system, four 10 second vapor puffs (at 2 second intervals) were delivered every 5 minutes. For the SMOK / SSV-3 apparatus, one 6-second vapor puff was delivered every 5 minutes. These parameters have been developed, in previous work, to generate similar vapor fill parameters and similar effects in rodent subjects.

2.4 Tissue Collection and Analysis

For these studies, animals were obtained, dosed and euthanized for tissue collection within 4-6 hours. Lobsters were exposed to THC vapor for 30 (N=3) or 60 (N=3) minutes, then removed from the chamber and rinsed with tap water. Thereafter, they were rapidly euthanized by transection of the thoracic nerve cord using heavy kitchen shears and then transection of the thorax behind the brain by a heavy chef's knife. Samples included the gills, claw

muscle obtained from proximal and distal aspects, anterior and posterior segments of tail muscle, a red membrane surrounding the claw muscle (N=2 per exposure), brain, heart, liver (N=2 for the 30-minute condition) and hemolymph. Hemolymph was allowed to coagulate to facilitate analysis as ng of THC per mg of tissue, as with the other tissues. For N=2 per exposure-duration group, one claw was cooked immediately after euthanasia by boiling it in water for 10 minutes, prior to collection of muscle tissue and the red membrane that surrounds it. Tissues were frozen (-80C) for storage until analysis was conducted. Tissue THC content was quantified using liquid chromatography/mass spectrometry (LC/MS) adapted from methods describe previously (Irimia et al., 2015; Lacroix and Saussereau, 2012). THC was extracted from brain tissue by homogenization in chloroform/ACN (Folch et al., 1957) containing 100 ng/ml of THC-d3 as internal standard (15:5:1) followed by centrifugation, decanting of the lower supernatant phase, evaporation and reconstitution in acetonitrile for analysis. Specifically, ~200-300 mg of tissue was homogenized in 1.5 mL of chloroform, 0.500 mL of acetonitrile and 0.100 mL of deuterated internal standard (100 ng/mL THC-d3; Cerilliant). Samples were centrifuged at 3000 RPM for 10 minutes, followed by decanting of the lower supernatant phase, evaporation using a speedvac, and reconstitution in 200 uL of an acetonitrile/methanol/water (2:1:1) mixture. Separation was performed on an Agilent LC1100 using a gradient elution of water and methanol (both with 0.2% formic acid) at 300 uL/min on an Eclipse XDB-C18 column (3.5um, 2.1mm x 100mm). THC was quantified using an Agilent MSD6180 single quadrupole with electrospray ionization and selected ion monitoring [THC (m/z=315.2) and THC-d3 (m/z=318.2)]. Calibration curves were generated each day at a concentration range of 0-200 ng/mL with observed correlation coefficients of 0.9990.

2.5 Locomotion

For these studies, animals (N=7; 3 F) were maintained in the laboratory for 4-21 days in chilled (~8°C) aquariums. Locomotor behavior was measured in an aquatic open field arena which consisted of 45.7 cm L x 31.4 cm W x 31.4 cm D (at the bottom) clear plastic bins placed on a light surface and filled to a 20 cm depth with chilled (~10°C) salt water. The session was recorded for 30 minutes with a camera (Logitech Model #C270) placed approximately 1 meter above the arena.

Video recording and movement analysis was conducted with ANY-Maze (Stoelting Co.) tracking software. Parameters of movement, including total time spent mobile (seconds), total distance traveled (meters) and speed (meters/second), were extracted from the video recordings.

2.6 Nociception

For these studies, animals were maintained in the laboratory for 4-21 days in chilled (~8°C) aquariums. Salt-water baths for the nociception assay were maintained at the target temperature (using placement of a beaker on a hot plate or water bath) and confirmed by thermometer immediately prior to each test. The investigator held the animal gently by the thorax and the tail or the tip of the antenna was inserted approximately 3 cm; the claws were inserted to a depth of approximately 5 cm. The latency to respond was recorded by stopwatch and a maximum 15 second interval was used as a cutoff for the assay. *Homarus* genus lobsters exhibit asymmetry of their claws with one larger (crusher) and one smaller (pincer, cutter) claw that can be on either the left or the right; feral experience appears to be necessary for proper development of the claw asymmetry as it is less pronounced in cultivated lobsters (van der Meeren and UKSNØY, 2000). This asymmetry produces a crusher muscle that is constituted of 100% slow fibers, whereas the cutter muscle exhibits only 90% fast fibers as assessed by ATPase staining and fast and slow motoneuron innervation (Govind, 1992). Thus, for this study the pincer and crusher claws were assessed independently. The order of assessment was always tail, claw, claw (pincer/crusher randomized in order) then antenna. The body part was inserted in the ambient (housing temperature, i.e., ~-10 °C) water bath for 5-10 seconds after each warm water assessment. The temperatures for assessment were "ambient", and then three warm temperatures (40°C, 44°C and 48°C); the order of testing of the warmer temperatures was in a counterbalanced order with at least two hours between assessments and no more than two tests per day. Lobsters were next assessed for the reaction of tail, crusher and pincer claws, and the antenna to insertion in 48°C water after vapor exposure to PG or THC for 30 or 60 minutes.

2.7 Data Analysis

Concentrations of THC in tissues were analyzed by ANOVA with the between-subjects factors of inhalation duration and within-subjects factor of body tissue. The nociceptive (latency) and locomotor (speed, distance, time mobile) data were assessed using within-subjects factors of vapor condition (PG vs THC), time (bin) after vapor exposure and body part (nociception). Any significant effects were followed with post-hoc analysis using Tukey correction for all multi-level, and Sidak correction for any two-level, comparisons. All analysis used Prism 9 for Windows (v. 9.1.0; GraphPad Software, Inc, San Diego CA).

3. Results:

3.1 Tissue THC levels

Tissue concentrations of THC depended on the duration of vapor exposure with significantly more THC produced by 60 minutes of vapor exposure (**Figure 2**), as confirmed with a main effect of exposure duration in the mixed-effects analysis [$F_{(1, 4)} = 16.10$; $P<0.5$]. Samples of gill were analyzed and exhibited 6,730 (SEM: 1,099) and 6,441 (SEM: 2,390) ng/g THC amounts in the 30- and 60-minute exposure conditions respectively. This was much higher than any other tissue and is consistent with vapor containing high levels of THC collecting on the outside of the gill structure.
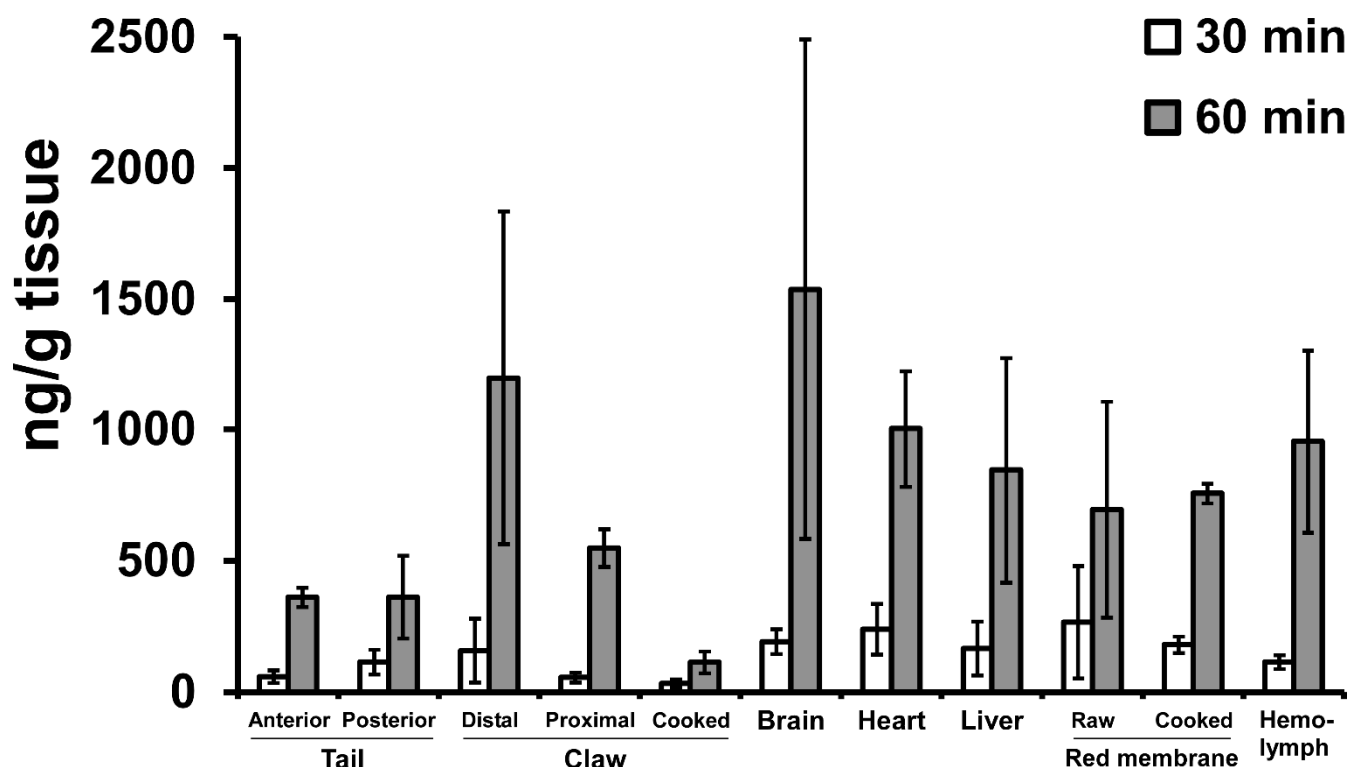
Figure 2: Mean (N=2-3; ±SEM) THC concentrations in various tissues after 30 or 60 minutes of exposure to THC vapor.

3.2 Locomotion

Lobsters spent more time mobile than immobile when in the test arena, with mean Time Mobile values in excess of 600 seconds within each 15-minute half of the session after PG exposure (**Figure 3**). Separate two-way ANOVAs for each locomotor measure confirmed a significant effect of time bin on Speed [$F_{(1, 6)} = 6.69$; $P<0.05$], Distance [$F_{(1, 6)} = 6.74$; $P<0.05$] and Time mobile [$F_{(1, 6)} = 15.25$; $P<0.01$]. These analyses also confirmed a significant effect of Vapor inhalation condition on Speed [$F_{(1, 6)} = 7.83$; $P<0.05$] and Distance [$F_{(1,}$

$6) = 8.68$; $P<0.05$]. The post hoc test confirmed that Speed and Distance were lower after THC vapor exposure, compared with PG vapor exposure, for each half of the session.

3.3 Nociception

Distinct responses of tail, antenna and claw were observed following insertion in warm water but not in the ambient temperature water bath. The response following insertion of the tail consisted of two distinguishable responses. Sometimes, a

5
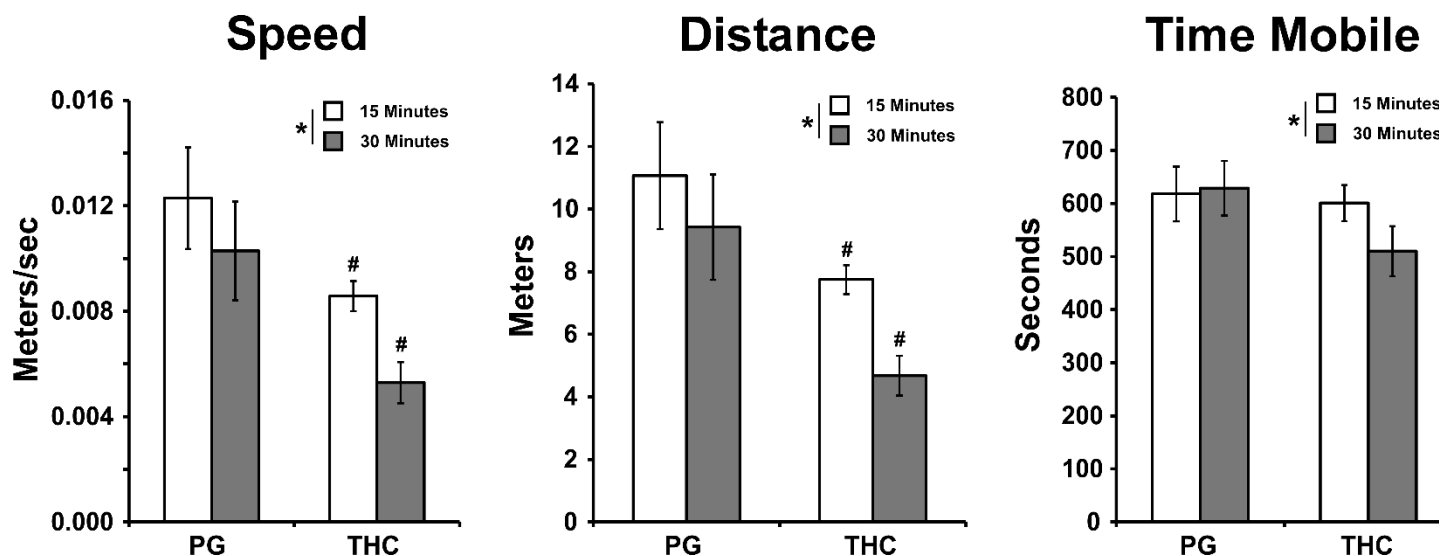
Figure 3: *Mean (N=7; ±SEM) locomotor behavior after 30 minutes of exposure to THC vapor. Parameters of movement Speed, Distance traveled and Time in which the animal was mobile are presented. A significant difference between the first and second half of the recording interval is indicated with * and a difference relative to the PG condition, within time bin, is indicated with #.*

reflexive and powerful contraction of the tail muscle was observed first (see **Supplementary Materials**). This is similar to the caridoid escape response described in crayfish, which is a complex behavior mediated by one lateral giant interneuron and one medial giant interneuron (Wiersma and Ikeda, 1964). In other cases, the lobster initiated distinct movements of legs and claws, this often preceded the powerful tail contraction. Thus, the tail assay was scored with two latency values, the very first reaction

of any type (tail reaction) and the tail contraction if it occurred. For analysis, the time of first overt response ("tail reaction") was used. The ANOVA confirmed a significant effect of body part [F (2.495, 22.45) = 11.94; P<0.0005] and water temperature [F (2.668, 24.01) = 30.89; P<0.0001] on withdrawal latency. The post hoc test of the marginal means confirmed that withdrawal latencies in ambient temperature differed from all other temperatures, and latencies in each of 44°C and 48°C differed from
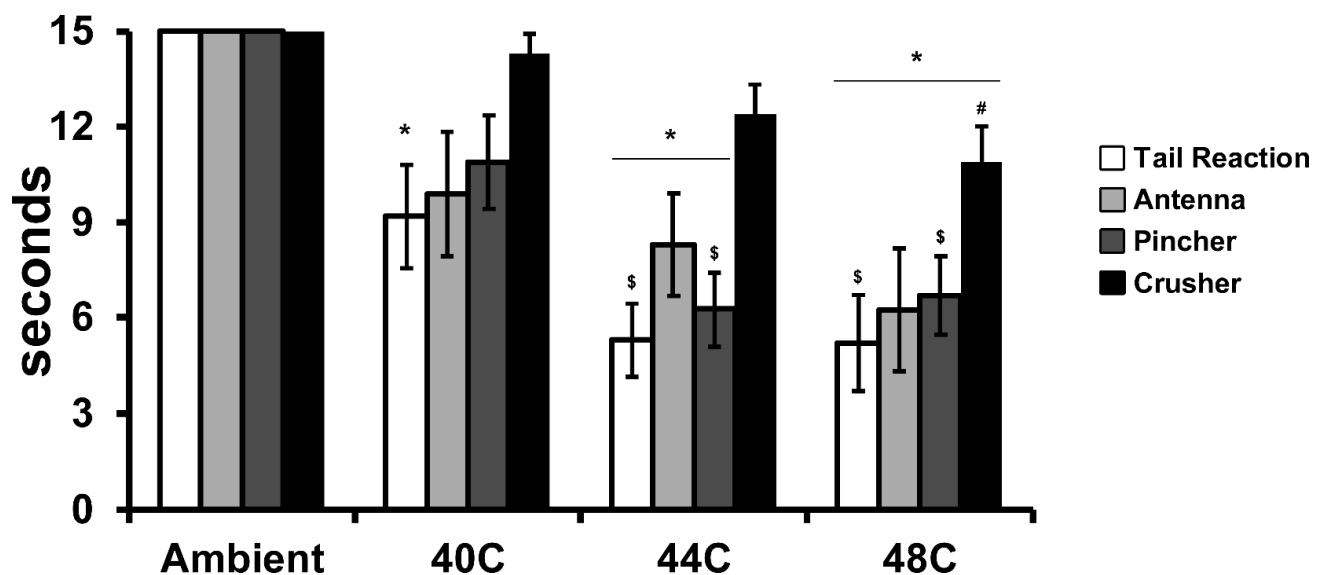
Figure 4: *Mean N=11 (3 F); latency to respond to the immersion in the water bath of the indicated temperature. A significant difference from ambient, within body part, is indicated with *, a difference from the 40°C condition, within body part with # and a difference from the Crusher claw, at a given temperature, with $.*

latencies observed after 40°C insertion. Post hoc of the marginal means for body part confirmed latency for the Crusher claw was slower than for each of the other body parts.
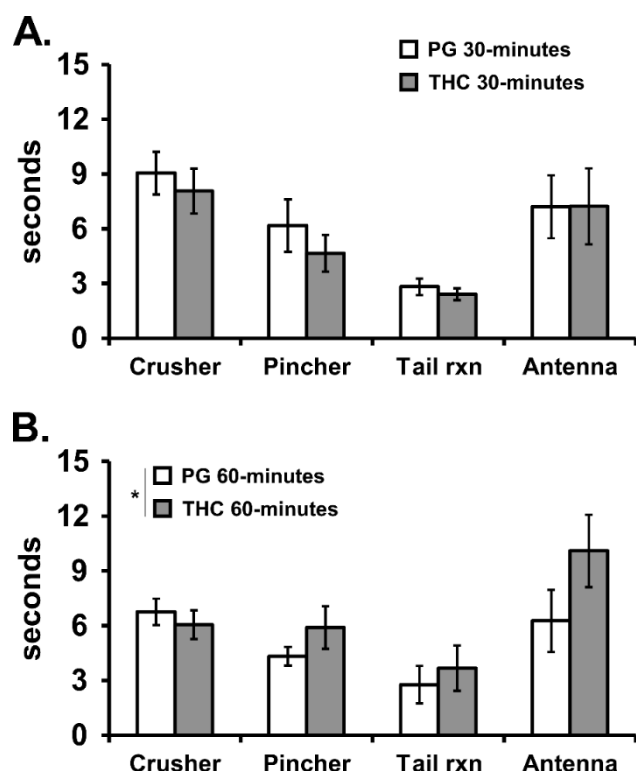
Figure 5. Mean (N=7; ±SEM) latency to react to immersion in 48°C water after A) 30 or B) 60 minutes of exposure to vapor from the propylene glycol (PG) vehicle or THC (100 mg/mL in the PG). Tail rxn = Tail reaction, i.e., the first defined movement. A significant difference between Vapor Conditions, across body part, is indicated with *.

The reaction to the 48°C water insertion was only marginally affected by vapor exposure to THC (**Figure 5**). The three-way ANOVA confirmed a significant effect of body part [F (3, 24) = 4.59; P=0.0112], and of the interaction of Vapor Condition with Exposure Duration [F (1, 24) = 6.68; P<0.05] on reaction latency. Follow-up two-way ANOVAs for each exposure duration confirmed a significant effect of Body Part [F (1.937, 11.62) = 8.03; P<0.01] after 30 minutes of exposure (but no effect of Vapor Condition) and a significant effect of Body Part [F (1.761, 10.57) = 6.28; P<0.05] and the interaction of Body Part with Vapor Condition [F (2.477, 14.86) = 4.19; P<0.05] after 60 minutes of exposure. The post-hoc test failed to confirm any significant difference associated with Vapor Condition for any individual body part.

4. Discussion:

The primary finding of this study was to confirm that vapor exposure of Maine lobsters (*Homarus americanus)* to Δ9-tetrahydrocannabinol (THC), using an e-cigarette based system, produces tissue levels of THC in a dose (time of vapor exposure) dependent manner. THC was confirmed in the hemolymph (the "blood" of the lobster), claw and tail muscle, brain, heart and liver (**Figure 2**). This wide distribution across body tissues is consistent with respiratory uptake, i.e. via the gills with distribution by the hemolymph circulation of the lobster. This conclusion is further supported by the much higher amount of THC that was associated with the gill tissue, consistent with a limited uptake by the respiration system of the lobster.

The THC exposure also had behavioral consequences, since locomotor activity was significantly reduced after exposure to THC vapor compared with exposure to the vehicle vapor (**Figure 3**). Hypolocomotion is a canonical feature of THC exposure in rats and mice, at least at higher doses, thereby confirming a similarity of effect across vertebrate and invertebrate organisms. This may be specifically related to a report of THC altering the amplitude of excitatory potentials at the lobster neuromuscular junction in a concentration dependent manner (Turkanis and Karler, 1988). Overall, however, it confirms that the levels of THC achieved by only 30 minutes of vapor exposure were behaviorally significant. One caveat for the locomotor studies is that the arena was not as large compared with the size of a lobster as the similar ratio for typical open field studies conducted in rodents. Similarly, the water depth was limited to that necessary to cover the lobster to facilitate the video tracking for this initial investigation. Nevertheless, the animals were able to express movement, turn around, change direction, etc and traveled about 20 meters after the vehicle exposure condition. It would be of interest in future studies to assess locomotor behavior in a larger arena or to assess behavior in a deeper aquatic environment.

In the nociception experiment, lobsters were observed to respond to warm water immersion of claw, tail or antenna in a temperature-dependent manner (**Figure 4**). This provides evidence of thermal nociception in the lobster for the first time (Walters, 2018), and is consistent with prior work which has shown thermal nociception in crayfish, using a warm (54°C) metal stimulus on the claw and antenna (Puri and Faulkes, 2015). No response to immersion in maintenance temperature (~10°C) salt

water was observed in this study, using the 15 sec cutoff. (In initial/pilot studies, there was also no response observed at laboratory ambient temperature of ~22-24°C). At temperatures from 40-48°C, however, the lobsters made distinct motor responses upon immersion of the tail, the claws or the antenna. Tail immersion resulted in a clear response of legs and claws and/or a strong flick of the tail. This latter is the escape response of lobsters (and crayfish) and confirms the noxiousness of the stimulus. Immersion of the claws or antenna resulted in a distinct movement to remove the appendage from the water. Temperature dependent differences in response latency were observed for the warm water challenges, with 40°C apparently less noxious than 44 or 48°C. This graded response is what is observed with a similar nociceptive assay in rats (Javadi-Paydar et al., 2018) and further enhances confidence in the specificity of the response to the noxious stimulus. The pronounced difference in sensitivity between the crusher and pincer claws provides another important validation of the model. Prior reports have focused on claw morphology and muscle fiber type (van der Meeren and UKSNØY, 2000) and this extends this by demonstrating a clear behavioral insensitivity of the crusher. Finally, the effect of THC vapor exposure on thermal nociception was minimal under the tested conditions. Surprisingly, despite the locomotor effect of 30 min of THC vapor exposure, there was no impact relative to vehicle vapor exposure on the latency of the response to warm water immersion (of any body part). It required 60 minutes of exposure to THC to produce any significant effect (**Figure 5B**), which was very small in magnitude. Although THC has limited anti-nociceptive impact in rodents relative to an opioid (Nguyen et al., 2019) and has a limited dose-effect range due to this low ceiling, it is typically more robust in rodents than what was observed here.

In conclusion, these data confirm a method for studying the effects of aerosol THC exposure in a lobster model. Duration-dependent levels of THC were observed in the species' tissues and a reduction in locomotor behavior was produced. The animals also responded in a temperature-dependent manner to the immersion of tail, claw or antenna in a hot water bath, indicating thermal nociception. This latter conclusion was further enhanced by the observation of differential sensitivity in the pincer and crusher claws. Thus, the assertions of the restauranteur that cannabinoids could be introduced into the lobster by atmospheric exposure (Grunewald, 2019; Hinckley, 2018; Stone, 2018),

and that this would be in sufficient amount to induce behavioral effect is supported. The impact of THC on thermal nociception was, however, minimal. Further experimentation would be required to fully investigate other behavioral outcomes, including anxiety-like measures.

Author Contributions

AG contributed to the overall design of the studies, to assay development, created the locomotor assessment approaches and video analysis, collected data, and provided initial data analysis. KMC contributed to behavioral assay development and study design, euthanized lobsters and collected tissues, designed and implemented housing and husbandry procedures, collected data and performed initial data analysis. MT and RT made critical contributions during assay development, including the critical pincer/crusher claw distinction, collected data and assisted with assessing relevant literature. TMK conducted tissue THC assays. JDN assisted with vapor exposure apparatus, overall experimental design and data collection. MAT secured the animal protocol approvals, euthanized lobsters, collected tissues, designed and conducted the cooking assay, contributed to the overall design of studies, conducted statistical analysis, figure creation and

initial drafting of the manuscript. All authors reviewed drafts and approved the manuscript.

Literature Cited

Beardsley, P.M., Scimeca, J.A., Martin, B.R., 1987. Studies on the agonistic activity of delta 9-11-tetrahydrocannabinol in mice, dogs and rhesus monkeys and its interactions with delta 9-tetrahydrocannabinol. J Pharmacol Exp Ther 241(2), 521-526.

Couillard, C.M., Burridge, L.E., 2015. Sublethal exposure to azamethiphos causes neurotoxicity, altered energy allocation and high mortality during simulated live transport in American lobster. Ecotoxicol Environ Saf 115, 291-299.

Eisen, J.S., Marder, E., 1984. A mechanism for production of phase shifts in a pattern generator. J Neurophysiol 51(6), 1375-1393.

Fitzgerald, K.T., Bronstein, A.C., Newquist, K.L., 2013. Marijuana poisoning. Top Companion Anim Med 28(1), 8-12.

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226(1), 497-509.

Forgan, L.G., Tuckey, N.P., Cook, D.G., Jerrett, A.R., 2014. Temperature effects on metabolic rate and cardiorespiratory physiology of the spiny rock lobster (Jasus edwardsii) during rest, emersion and recovery. J Comp Physiol B 184(4), 437-447.

Govind, C.K., 1992. Claw asymmetry in lobsters: case study in developmental neuroethology. J Neurobiol 23(10), 1423-1445.

Grunewald, W., 2019, Every Lobster Must Get Stoned. Down East. Down East Magazine. https://downeast.com/cook-lobster-with-cannabis/

Hinckley, S., 2018, "Hot box" lobsters touted. Mount Desert Islander. Ellsworth American. https://www.mdislander.com/maine-news/hot-box-lobsters-touted

Huber, R., Imeh-Nathaniel, A., Nathaniel, T.I., Gore, S., Datta, U., Bhimani, R., Panksepp, J.B., Panksepp, J., van Staaden, M.J., 2018. Drug-sensitive Reward in Crayfish: Exploring the Neural Basis of Addiction with Automated Learning Paradigms. Behav Processes 152, 47-53.

Huber, R., Panksepp, J.B., Nathaniel, T., Alcaro, A., Panksepp, J., 2011. Drug-sensitive reward in crayfish: an invertebrate model system for the study of SEEKING, reward, addiction, and withdrawal. Neurosci Biobehav Rev 35(9), 1847-1853.

Imeh-Nathaniel, A., Rincon, N., Orfanakos, V.B., Brechtel, L., Wormack, L., Richardson, E., Huber,

R., Nathaniel, T.I., 2017. Effects of chronic cocaine, morphine and methamphetamine on the mobility, immobility and stereotyped behaviors in crayfish. Behav Brain Res 332, 120-125.

Irimia, C., Polis, I.Y., Stouffer, D., Parsons, L.H., 2015. Persistent effects of chronic Delta9-THC exposure on motor impulsivity in rats. Psychopharmacology (Berl) 232(16), 3033-3043.

Javadi-Paydar, M., Kerr, T.M., Harvey, E.L., Cole, M., Taffe, M.A., 2019. Effects of nicotine and THC vapor inhalation administered by an electronic nicotine delivery system (ENDS) in male rats. Drug Alcohol Depend 198, 54-62.

Javadi-Paydar, M., Nguyen, J.D., Kerr, T.M., Grant, Y., Vandewater, S.A., Cole, M., Taffe, M.A., 2018. Effects of Delta9-THC and cannabidiol vapor inhalation in male and female rats. Psychopharmacology (Berl) 235(9), 2541-2557.

Lacroix, C., Saussereau, E., 2012. Fast liquid chromatography/tandem mass spectrometry determination of cannabinoids in micro volume blood samples after dabsyl derivatization. J Chromatogr B Analyt Technol Biomed Life Sci 905, 85-95.

Marder, E., Eisen, J.S., 1984. Electrically coupled pacemaker neurons respond differently to same physiological inputs and neurotransmitters. J Neurophysiol 51(6), 1362-1374.

McMahon, L.R., Koek, W., 2007. Differences in the relative potency of SR 141716A and AM 251 as antagonists of various in vivo effects of cannabinoid agonists in C57BL/6J mice. Eur J Pharmacol 569(1-2), 70-76.

Nguyen, J.D., Aarde, S.M., Vandewater, S.A., Grant, Y., Stouffer, D.G., Parsons, L.H., Cole, M., Taffe, M.A., 2016. Inhaled delivery of Delta(9)-tetrahydrocannabinol (THC) to rats by e-cigarette vapor technology. Neuropharmacology 109, 112-120.

Nguyen, J.D., Creehan, K.M., Grant, Y., Vandewater, S.A., Kerr, T.M., Taffe, M.A., 2020. Explication of CB1 receptor contributions to the hypothermic effects of Delta(9)-tetrahydrocannabinol (THC) when delivered by vapor inhalation or parenteral injection in rats. Drug Alcohol Depend 214, 108166.

Nguyen, J.D., Grant, Y., Creehan, K.M., Hwang, C.S., Vandewater, S.A., Janda, K.D., Cole, M., Taffe, M.A., 2019. Delta(9)-tetrahydrocannabinol attenuates oxycodone self-administration under extended access conditions. Neuropharmacology 151, 127-135.

Puri, S., Faulkes, Z., 2015. Can crayfish take the heat? Procambarus clarkii show nociceptive

behaviour to high temperature stimuli, but not low temperature or chemical stimuli. Biol Open 4(4), 441-448.

Smith, M.A., 2020. Nonhuman animal models of substance use disorders: Translational value and utility to basic science. Drug Alcohol Depend 206, 107733.

Stone, S., 2018, Maine Asks Restaurant To Stop Giving Lobsters Cannabis Before Boiling Them. NPR. National Public Radio. https://www.npr.org/sections/thesalt/2018/09/25/651466068/maine-asks-restaurant-to-stop-giving-lobsters-cannabis-before-boiling-them

Taffe, M.A., 2012. Delta9-Tetrahydrocannabinol attenuates MDMA-induced hyperthermia in rhesus monkeys. Neuroscience 201, 125-133.

Taffe, M.A., Creehan, K.M., Vandewater, S.A., 2015. Cannabidiol fails to reverse hypothermia or locomotor suppression induced by Delta(9) - tetrahydrocannabinol in Sprague-Dawley rats. Br J Pharmacol 172(7), 1783-1791.

Taffe, M.A., Creehan, K.M., Vandewater, S.A., Kerr, T.M., Cole, M., 2021. Effects of Delta(9)-tetrahydrocannabinol (THC) vapor inhalation in Sprague-Dawley and Wistar rats. Exp Clin Psychopharmacol 29(1), 1-13.

Thirumalai, V., Marder, E., 2002. Colocalized neuropeptides activate a central pattern generator by acting on different circuit targets. J Neurosci 22(5), 1874-1882.

Thompson, G.R., Rosenkrantz, H., Schaeppi, U.H., Braude, M.C., 1973. Comparison of acute oral toxicity of cannabinoids in rats, dogs and monkeys. Toxicol Appl Pharmacol 25(3), 363-372.

Tseng, A.H., Craft, R.M., 2001. Sex differences in antinociceptive and motoric effects of cannabinoids. Eur J Pharmacol 430(1), 41-47.

Turkanis, S.A., Karler, R., 1988. Changes in neurotransmitter release at a neuromuscular junction of the lobster caused by cannabinoids. Neuropharmacology 27(7), 737-742.

van der Meeren, G.I., UKSNØY, L.E., 2000. A comparison of claw morphology and dominance between wild and cultivated male European lobster. Aquaculture International 8, 77-94.

Walters, E.T., 2018. Nociceptive Biology of Molluscs and Arthropods: Evolutionary Clues About Functions and Mechanisms Potentially Related to Pain. Front Physiol 9, 1049.

Wiersma, C.A., Ikeda, K., 1964. Interneurons Commanding Swimmeret Movements in the Crayfish, Procambarus Clarki (Girard). Comp Biochem Physiol 12, 509-525.

Wiley, J.L., 2003. Sex-dependent effects of delta 9-tetrahydrocannabinol on locomotor activity in mice. Neurosci Lett 352(2), 77-80.

Wiley, J.L., O'Connell M, M., Tokarz, M.E., Wright, M.J., Jr., 2007. Pharmacological effects of acute and repeated administration of Delta(9)-tetrahydrocannabinol in adolescent and adult rats. J Pharmacol Exp Ther 320(3), 1097-1105.