

1 **Behavioral differences among domestic cats in the response**
2 **to cat-attracting plants and their volatile compounds reveal a**
3 **potential distinct mechanism of action for actinidine**

6 **Response of cats to cat-attracting plants and their active volatiles**

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19 *Valeriana officinalis*, catnip, silver vine, Tatarian honeysuckle, nepetalactone, dihydroactinidiolide,
20 iridomyrmecin, scent, enrichment, fragrance

21 Abstract

22

23 It has been known for centuries that cats respond euphorically to *Nepeta cataria* (catnip). Recently, we
24 have shown that *Lonicera tatarica* (Tatarian honeysuckle), *Actinidia polygama* (silver vine) and *Valeriana*
25 *officinalis* (valerian) can also elicit this “catnip response”. The aim of this study was to learn if the behavior
26 seen in response to these plants is similar to the response to catnip. Furthermore, we studied if these
27 responses are fixed or if there are differences between cats. While nepetalactone was identified decades
28 ago as the molecule responsible for the “catnip response”, we know that this volatile is found almost
29 exclusively in catnip. Therefore, we also aimed to identify other compounds in these alternative plants
30 that can elicit the blissful behavior in cats.

31 Bioassays with 6 cats were performed in a stress-free environment, where 6 plants and 13 single
32 compounds were each tested for at least 100 and 17 hours, respectively. All responses were video
33 recorded and BORIS software was used to analyze the cats’ behavior.

34 Both response duration and behavior differed significantly between the cats. While individual cats had
35 preferences for particular plants, the behavior of individual cats was consistent among all plants. About
36 half a dozen lactones similar in structure to nepetalactone were able to elicit the “catnip response”, as
37 were the structurally more distinct molecules actinidine and dihydroactinidiolide. Most cats did not
38 respond to actinidine, whereas those who did, responded longer to this volatile than any of the other
39 secondary plant metabolites, and different behavior was observed. Interestingly, dihydroactinidiolide was
40 also found in excretions and secretions of the red fox, making this the first report of a compound
41 produced by a mammal, that can elicit the “catnip response”. A range of different cat-attracting
42 compounds was detected by chemical analysis of plant materials but differences in cat behavior could not
43 be directly related to differences in chemical composition of the plants. Together with among other results
44 of habituation / dishabituation experiments, this indicates that additional cat-attracting compounds may be
45 present in the plant materials that remain to be discovered.

46 Collectively, these findings suggest that both the personality of the cat and genetic variation in the genes
47 encoding olfactory receptors may play a role in how cats respond to cat-attracting plants. Furthermore,
48 the data suggest a potential distinct mechanism of action for actinidine.

49 Introduction

50

51 Cats are lured by the volatiles of several plant species and unlike any other animal they demonstrate
52 what appears to be blissful behavior in response to smelling them. Of these plants, the species *Nepeta*
53 *cataria* (catnip) and *Actinidia polygama* (silver vine) are the best-known to elicit such a response. The
54 former is commonly used by cat caregivers in Europe and North America, while the latter is more popular
55 in Asia, where it is also known as matatabi. After sniffing these plants, head rubbing and rolling over are
56 typically observed, and this behavior is generally referred to as the “catnip response” (Bol et al. 2017,
57 Todd 1963). While the joyful effects of some plants from the genus *Nepeta* on cats has been known to
58 humans for centuries (Ray 1660, Salmon 1710), it is still unclear if there is a biological reason for the
59 response of cats to this select group of plants. Felines are believed not to be the intended recipients of
60 the allomones produced by these plants. This unique response of cats appears to be fortuitous, since
61 plants produce these secondary metabolites to protect themselves against phytophagous or parasitic
62 insects. The cat-attracting compounds synthesized by a small number of species within the plant kingdom
63 are identical or closely related to insect pheromones or allomones (Beran et al. 2019, Eisner 1964).
64 Insects release these chemicals when in danger (Ho and Chow 1993, Kanehisa, Tsumuki and Kawazu
65 1994) and for this reason it is assumed plants produce and release these chemicals to send a warning
66 message to phytophagous insects (Ebrahim et al. 2015, Stökl et al. 2012, Welzel et al. 2018). Recently,
67 Nadia Melo and her colleagues revealed the molecular mechanism by which the iridoid nepetalactone
68 repels insects (Melo et al. 2021).
69 Nepetalactone, found in *Nepeta cataria*, was the first compound identified as being able to elicit the catnip
70 response (McElvain, Bright and Johnson 1941). Several other compounds similar in structure, have been
71 reported to have effects comparable to nepetalactone (Sakan et al. 1959, Sakan, Fujino and Murai 1960,
72 Sakan et al. 1965, Johnson and Waller 1971, Scaffidi et al. 2016), but bioassays with cats were not
73 performed. However, behavior analogous to the “catnip response” was observed when felines were
74 exposed to *A. polygama*, *Lonicera tatarica* (Tatarian honeysuckle) and *Valeriana officinalis* (valerian) root,
75 all containing little to no nepetalactone (Bol et al. 2017). Those results suggest other compounds are also
76 able to elicit the “catnip response”. Unpublished work (doctoral dissertation) by Nelson and Wolinsky

77 done more than 50 years ago provided some more insight into which compounds might be able to elicit
78 the “catnip response” in domestic cats, which included several lactones (nepetalactone,
79 epinepetalactone, iridomyrmecin, isoiridomyrmecin, dihydronepetalactone, isodihydronepetalactone,
80 neonepetalactone) and matatabiether (Nelson 1968). Results from a recent study by Reiko Uenoyama *et*
81 *al.*, that were published while this manuscript was in preparation, indicated that domestic cats respond to
82 a variety of lactones (nepetalactone, iridomyrmecin, isoiridomyrmecin, dihydronepetalactone,
83 isodihydronepetalactone) as well as nepetalactol (Uenoyama *et al.* 2021). Most of what is known about
84 the behavior of domestic cats seen in response to cat-attracting plants originates from a limited number of
85 studies where only catnip was used (Todd 1962, Todd 1963, Hill *et al.* 1976, Palen and Goddard 1966).
86 With this study we tried to answer several questions, including the following. (i) We wanted to know if the
87 cats’ behavior to other known cat-attracting plants is the same as to catnip. To this end, we performed
88 comprehensive behavioral analysis of 6 domestic cats in response to *Actinidia polygama* (silver vine),
89 *Lonicera tatarica* (Tatarian honeysuckle), *Valeriana officinalis* (valerian) and the arcane *Acalypha indica*
90 (Indian nettle), and compared these responses to the behavior seen in response to *Nepeta cataria*
91 (catnip). (ii) In addition, we wanted to learn if the “catnip response” is a fixed, predictable, biological
92 response to these cat-attracting plants, or if there is variation in the response between cats. Therefore,
93 we also compared the observed behavior between the 6 cats. (iii) Furthermore, we wanted to know which
94 single compounds the cats respond to and understand which features of these molecules are responsible
95 for the response. For this reason, we studied the response of domestic cats to all lactones tested by
96 Uenoyama *et al.*, but also included indole, neonepetalactone, isoneonepetalactone, and the structurally
97 more distinct actinidine (a pyridine) and dihydroactinidiolide (a furanone), both known to be present in *A.*
98 *polygama* (Sakan *et al.* 1965, Sakan, Isoe and Hyeon 1967, Sakan *et al.* 1969, Bol *et al.* 2017). Not only
99 did we test if cats responded to these compounds from different classes, but (iv) we were also interested
100 to see if the cats’ behavior varies between the different compounds or between cats. After video recording
101 the responses of 6 domestic cats to 5 different plants and 13 single compounds on 72 days between the
102 summer of 2018 and the winter of 2020, we analyzed 470 responses to plants, totaling over 8 hours of
103 response time, and 217 responses to single compounds, totaling over 2.5 hours of response time. Of
104 these, the behavior of 179 responses (88 to plants and 91 to single compounds), totaling over 77 and 80

105 minutes, respectively, were analyzed in detail using behavioral analysis software. In addition to the
106 behavioral studies, (v) we quantified the amount of the various single compounds in the plants that were
107 used in this study in an attempt to correlate these with the duration and behavior seen in response to the
108 plants.

109 **Materials and methods**

110

111 **Ethics**

112 No cats were hurt or distressed for this study, nor were they ever forced to act, respond, or behave in any
113 way. All research with cats was non-invasive and did not involve pharmacological, medical, or surgical
114 intervention. All participating cats were adopted and are permanent residents of Cowboy Cat Ranch,
115 living together with authors SB and EMB. The study protocol was approved by the Cowboy Cat Ranch
116 Institutional Animal Care and Use Committee (IACUC). Cowboy Cat Ranch was registered with the USDA
117 as a research facility (registration number 74-R-0224, ID no. 502147) during the time of the study (2018 –
118 2020). The research facility and the cats were inspected annually by the Cowboy Cat Ranch IACUC and
119 the USDA Animal and Plant Health Inspection Service.

120

121 **Study population**

122 Six healthy, neutered, adult, domestic short-haired cats (**Table 1**) participated in this study that
123 commenced in June 2018 and ended in December 2020. All cats were adopted from a local shelter, with
124 cats N, O and V from the same litter. In December of 2018 cat H needed to be separated from the other
125 cats for medical reasons and was therefore not exposed to *V. officinalis* and the single compounds. All
126 cats were seen by a veterinarian (Babcock Hills Veterinary Hospital in San Antonio, TX, USA) for routine
127 veterinary care (physical examination, blood tests, vaccinations, dental cleaning and dental X-rays) at
128 least once a year, were treated once a month with Catego (dinotefuran, fipronil and pyriproxyfen) for flea
129 and tick control, and received milbemycin oxime for heartworm prevention and intestinal parasite control
130 once a month. In November 2020 cat A was diagnosed with hyperthyroidism and treated with radioiodine
131 that same month.

132

133 **Study environment**

134 All experiments were done in a stress-free setting. Cowboy Cat Ranch is the permanent home of all the
135 cats who participated in this study, as well as the authors and researchers SB and EMB. It is a one-story
136 house with 195 m² indoor living space and 51 m² enclosed outdoor space on 4.1-hectare privately-owned

137 land, with little to no distraction. The testing area consisted of a 6.3 m² (3.3 × 1.9 m) piece of vinyl sheet
138 (**Supplementary Figure 1A**) placed in the center of the floor of a 45 m² (8.2 × 5.5 m²) open room that
139 was recorded continuously when an olfactory test sample was made available to the cats. The cats were
140 motivated to spend time in the open room with the testing area by temporarily restricting their living space
141 (not allowing access to other rooms or outdoor enclosures), offering treats close to, but not at, the testing
142 area at set times, or by being present in the room where the olfactory samples were available. The latter
143 two strategies were only employed when the single compounds were tested. For the safety of the cats,
144 the IACUC required at least one human to be present during the testing of these compounds to actively
145 look for any potential adverse reactions (none were observed), since felines have not been previously
146 exposed to several of these before. When the tested plants were available for the cats (10 days per plant,
147 10 hours per day), no humans were present. The minimum living space for the cats during the testing
148 period was 120 m² and contained twelve large (about 2 m tall) cat trees, more than 37 m of wall shelves,
149 multiple cat beds and comforters to provide vertical space and hiding places. None of the cats were ever
150 forced to be at a certain location, act, respond or behave in any way. The indoor temperature was
151 maintained constant at 20 – 23°C and all rooms were illuminated when it was dark outside. The cats were
152 fed canned food four to six times a day, had continuous access to running and standing water, multiple
153 litter boxes and fresh (no older than 14 days after seeding) oat grass.

154

155 **Plants**

156 Five different plant species were used in this study: *Nepeta cataria* or catnip, *Acalypha indica* or Indian
157 nettle, *Actinidia polygama* or silver vine, *Lonicera tatarica* or Tatarian honeysuckle, and *Valeriana*
158 *officinalis* or valerian (**Table 2, Supplementary Figure 1B-G**). *Actinidia polygama* 'Hot Pepper' (female)
159 and 'Pavel' (male) varieties were purchased as one gallon-size plants in 2017. Before collecting leaves
160 and woody stems in October/November 2020, the plants had been growing in Mico, Texas, USA for three
161 and a half years. Leaves and stems from these plants were dried at room temperature and 30-50%
162 humidity for one to two weeks. The stem used for testing was woody, 15 cm long, and had a diameter of
163 1 cm (similar to what is commercially available). With the exception of *A. polygama* stem, 15 g of each
164 plant material was offered to the cats. *A. indica* roots were collected from Christmas Island, Australia as

165 described previously (Scaffidi et al. 2016). The roots were washed free of soil material and lyophilized
166 immediately after collection and stored in vacuum sealed bags until use. All plant materials were stored
167 airtight at room temperature, away from direct sunlight.

168

169 **Single compounds**

170 Thirteen different single compounds were used in this study to test if and how domestic cats responded to
171 them (**Table 3**): **(1)** nepetalactone ((4aS,7S,7aR)-4,7-dimethyl-5,6,7,7a-tetrahydrocyclopenta[c]pyran-
172 1(4aH)-one), **(2)** epinepetalactone ((4aS,7S,7aS)-4,7-dimethyl-5,6,7,7a-tetrahydrocyclopenta[c]pyran-
173 1(4aH)-one), **(3)** dihydronepetalactone ((4S,4aR,7S,7aR)-4,7-dimethylhexahydrocyclopenta[c]pyran-
174 1(3H)-one), **(4)** isodihydronepetalactone ((4R,4aR,7S,7aR)-4,7-dimethylhexahydrocyclopenta[c]pyran-
175 1(3H)-one), **(5)** neonepetalactone ((4S,4aR)-4,4a,5,6-tetrahydro-4,7-dimethylcyclopenta[c]pyran-1(3H)-
176 one), **(6)** isoneonepetalactone ((4R,4aR)-4,4a,5,6-tetrahydro-4,7-dimethylcyclopenta[c]pyran-1(3H)-one),
177 **(7)** iridomyrmecin ((4S,4aS,7S,7aR)-4,7-dimethylhexahydrocyclopenta[c]pyran-3(1H)-one), **(8)**
178 isoiridomyrmecin ((4R,4aS,7S,7aR)-4,7-dimethylhexahydrocyclopenta[c]pyran-3(1H)-one), **(9)** actinidine
179 ((S)-4,7-dimethyl-6,7-dihydro-5H-cyclopenta[c]pyridine), **(10)** dihydroactinidiolide ((R)-4,4,7a-trimethyl-
180 5,6,7,7a-tetrahydrobenzofuran-2(4H)-one), **(11)** indole, menthol, and methyl salicylate.
181 Dihydroactinidiolide (**10**) and indole (**11**) were purchased from AK Scientific (#J10744 and #I908,
182 respectively). Compounds **1** and **9** (Beckett, Beckett and Hofferberth 2010), **2 – 4** (Scaffidi et al. 2016), **5**
183 – **6** (Enders and Kaiser 1997) and **7 – 8** (Stepanov and Veselovsky 1997) were all synthesized according
184 to literature procedures and shipped from Australia to the USA at room temperature in two clear glass
185 vials with Teflon screw caps, each containing approximately 5 mg of material accurately weighed. Upon
186 arrival, the vials were stored in the dark at 4°C. Immediately prior to testing the compounds were
187 dissolved in diethyl ether (Acros #448421000) at a concentration of 1 mg/mL using a Gilson Microman E
188 M1000E positive displacement pipet with 1 mL capillary pistons. The concentration of 1 mg/mL is
189 equivalent to about 6 mM for the lactones, 7 mM for actinidine, 5.5 mM for dihydroactinidiolide, 6.5 mM for
190 menthol and methyl salicylate, 4 mM for civetone and 8.5 mM for indole. While we did not normalize
191 based on the molecular weight of the compounds, three different amounts of each compound were
192 tested: 33 µg, 100 µg, 300 µg and 900 µg, equivalent to 33, 100, 300 and 900 µL of solution. These

193 amounts were chosen in the absence of information about the lower level of detection in domestic cats
194 and were somewhat arbitrary. While Nelson and Wolinsky tested 3,000 – 5,000 µg (Nelson 1968), we
195 decided to start with the lowest amount that we could reliably pipette and increase the volume (and hence
196 the amount of the compound) during the day or subsequent testing on another day to rule out that cats
197 did not respond because the amount used was too low. 50 – 400 µg of iridoids was used in the study led
198 by Masao Miyazaki (Uenoyama et al. 2021), who published the results while this manuscript was in
199 preparation. We did not screen different amounts with the intent to establish a dose response
200 relationship, since this would be complicated by both fluctuating activity levels of domestic cats during the
201 day (typically less active in the afternoon). Furthermore, previous exposures may have an effect on the
202 subsequent testing of higher amounts of the compounds, especially when different amounts are tested on
203 the same day. Since there was no information available on the stability of these compounds when
204 dissolved in diethyl ether, the goal was to test them immediately. This limited us to two testing days.
205 However, a limited amount of the compounds (neonepetalactone and isoneonepetalactone: one vial with
206 5 mg) and the need for additional testing (neonepetalactone: technical problems with recording; *trans-cis*-
207 nepetalactone and actinidine: assuring that lack of response was not due to limited exposure time)
208 required us to store compounds dissolved in diethyl ether for short periods of time. Neonepetalactone and
209 isoneonepetalactone were stored in the dark at 4°C for 4 days before 100 µg, 300 µg and 900 µg were
210 tested. Neonepetalactone had to be stored for a further 6 days because of technical issues while
211 recording. Testing *trans-cis*-nepetalactone and actinidine on more than two days was not anticipated, but
212 motivated by the results of the first two testing days (absence of response by multiple cats). After the
213 second test day, *trans-cis*-nepetalactone dissolved in diethyl ether was stored for one and a half months
214 at room temperature. Actinidine dissolved in diethyl ether was stored in the dark but at fluctuating
215 temperatures, ranging from freezing to room temperature and was used once to test 900 µg after two
216 weeks of storage. Actinidine used to test on the 4th and 5th day was a new shipment from Australia, which
217 was re-purified (silica chromatography) prior to shipping because some degradation (browning) of the
218 original stock (that was stored neat at 4°C) was noticed. In addition to the compounds mentioned above,
219 a small amount of *trans*-dihydronepetalactone ((4S,4aR,7S,7aS)-4,7-
220 dimethylhexahydrocyclopenta[c]pyran-1(3H)-one) and *trans*-isodihydronepetalactone ((4R,4aR,7S,7aS)-

221 4,7-dimethylhexahydrocyclopenta[c]pyran-1(3H)-one) was used for quantitative analysis and prepared by
222 reducing epinepetalactone (**2**) in a similar fashion to the preparation of dihydronepetalactone (**3**) and
223 isodihydronpetalactone (**4**) from nepetalactone (**1**) (Scaffidi et al. 2016).

224

225 **Testing procedures**

226 Cats were familiarized with the vinyl sheet and their altered environment for at least one month prior to
227 the start of the study. Thin, fibrous, porous, polyester socks with 2 – 3% spandex were used as carriers of
228 the olfactory material. The testing area accommodated a maximum of four socks / samples at the same
229 time. Socks were mounted near each corner of the testing area using twine in a way that allowed for
230 some movement (25 cm radius) of the sock, but eliminated cross-over contamination between samples or
231 controls (**Supplementary Figure 1A**) and prevented cats from moving the socks outside of the 4.5 m²
232 area captured by the camera. The olfactory samples were deliberately offered for many hours and on
233 multiple days to reduce the chance that a cat would not respond because of unawareness of the
234 presence of the olfactory stimulus, competition for the sample with another cat, or hindrance in any other
235 way by another cat. The vinyl sheet was cleaned with water and soap before and after every testing day.
236 Responses where the cats displayed behavior listed in the ethogram (see the paragraph *behavioral/*
237 *analysis* below) were considered catnip-responses or positive responses.

238

239 *Plants*

240 Five different plants plus a negative control (green tea) were tested between June 2018 and May 2019
241 (**Table 2, Supplementary Figure 2A**). Each sock contained 15 g of plant material. Samples and negative
242 controls were offered in duplicate to allow two cats to respond simultaneously. Samples were mounted
243 diagonally across so responding cats had enough space and would not disturb each other. Plant
244 materials were offered for 10 hours (between 9:30 and 19:30) on ten different days (Monday – Friday
245 only), within a total time period of five weeks: five days in the first two weeks and five days in the last two
246 weeks, separated by at least one full week (Monday – Sunday) of no exposure. Exposure was limited to
247 no more than three days in one week (Monday – Friday), never more than two days of exposure in a row,
248 but at least one consecutive two-day exposure per two-week period (**Supplementary Figure 2A**). To

249 avoid human presence affecting the outcome of the experiments no humans were present during these
250 tests. The schedule described above allowed for some flexibility, while keeping the testing conditions
251 highly similar for all plants tested. Different plant samples were offered no sooner than one week after the
252 previous testing was completed. The same socks and plant materials were used throughout the 5-week
253 testing period. On days and nights when the olfactory stimuli were not tested, they were stored in an air-
254 tight bag, away from direct sunlight, at room temperature. New socks were used when new plant
255 materials were tested. Since the tested plant samples could easily be identified by their smell, the
256 experiments with the plants were not performed blinded.

257

258 *Single compounds*

259 The single compounds were tested from December 2018 through to August 2020. Various volumes (33,
260 100, 300 or 900 µL) of compound dissolved in diethyl ether (final concentration of 1 µg/µL) were applied
261 on the outside of a sock. Equal amounts of diethyl ether were used for the negative control socks. The
262 diethyl ether was allowed to evaporate prior to mounting the socks. Compounds were tested on two
263 different days: 33 and 100 µg on day A for 1 and 4 hours in the afternoon, respectively, and 100, 300 and
264 900 µg on day B, all for 4 hours, starting at 7:30, ending at 19:30. All samples to be tested on the same
265 day (e.g., socks with 33 µL and 100 µL for day A) were prepared early in the morning, and were stored in
266 an air-tight bag away from direct sunlight at room temperature prior to use. There were always 3 days
267 between days A and B, to rule out responses on day A affecting responses on day B. While the recording
268 area had a capacity for 4 samples, no more than two different compounds were tested at the same time.
269 When possible, different combinations of compounds were tested on day A than on day B (e.g., *cis-trans*-
270 nepetalactone and iridomyrmecin on day A, but on day B *cis-trans*-nepetalactone was tested together
271 with dihydronepetalactone). We chose for this rotating setup to prevent false negative responses that
272 were the result of a strong preference for one compound over the other. Testing of *trans-cis*-
273 nepetalactone was repeated for this reason since this compound was offered in combination with
274 actinidine on both days, and cat A was extremely attracted to actinidine. We aimed to test the single
275 compounds as soon as possible after they were received as we were unsure about their stability.
276 However, not all the compounds were received at the same time and therefore some compounds were

277 tested together on both days A and B. Each sample was always accompanied by a negative control. To
278 comply with IACUC guidelines, at least one human was present during exposure tests with the single
279 compounds, since at the time the tests were conducted, no safety information was available for these
280 compounds. Socks containing higher amounts of the single compound were mounted on the same
281 location to prevent cross-contamination of the vinyl surface area. All compounds were coded and the
282 testing and analysis (response frequency and duration) were done blind.

283

284 **Behavioral analysis**

285 All responses of the cats to the plants or single compounds were video recorded. Behavioral analyses of
286 the responses were performed using the free, open-source software BORIS (version 7.9.19) (Friard and
287 Gamba 2016). The ethogram shown in **Table 4** was used for the video coding of the cats' behavior. The
288 video ethogram shows the behavior listed in the ethogram of the domestic cats who participated in this
289 study in response to the cat-attracting plants or single compounds (**Supplementary File 1**). Four
290 recordings are shown for each behavior listed in Table 4. The analysis using BORIS software was not
291 done blinded, since behavioral comparisons were done using recordings of responses with known
292 duration and hence after the quantitative analysis. Body position, biting, head rubbing, holding, licking and
293 raking were expressed as the percentage of the total response duration as determined by using BORIS.
294 Head shaking, rippling of the back, rolling on the side and twitching of the back were reported as events
295 per minute and plotted on a different Y axis to allow for better visualization of these behaviors and
296 discrimination of their frequency between cats or stimuli. Small discrepancies exist between the total
297 response time (used to calculate differences in response duration and timed with a stopwatch) and the
298 sum of the duration of all behaviors scored in BORIS. For the former, the total time the cat was
299 responding was used, and sometimes included aspects of the response that were not scored in BORIS
300 (e.g., stretching out while in lateral position in the middle of the response, but not actively engaging with
301 the test object, or, rarely seen, playfully running away from and towards the test object, swatting it when
302 passing by). In rare situations it was difficult to determine with certainty what the cat was doing, for
303 example, discriminating between head rubbing, licking or biting. When in doubt, the behavior was not
304 scored in BORIS.

305

306 **Habituation / dishabituation testing**

307 *Actinidia polygama*, *Lonicera tatarica* and *Nepeta cataria* (Frontier) were used to study habituation /
308 dishabituation of domestic cats to these cat-attracting plants (**Supplementary Figure 2B**). Half a gram
309 and 2.5 g dried *A. polygama* fruit gall powder, 2.5 g *L. tatarica* sawdust and 2.5 g dried *N. cataria* leaves
310 were available to the cats. Each plant material was tested on at least 10 consecutive days for either 2 or
311 12 hours (20:00 – 22:00 and 10:00 – 22:00, respectively). Two socks were available for each plant
312 material. No negative controls were used in these experiments. Prior to testing, no olfactory stimuli were
313 available to the cats for at least 2 weeks.

314

315 **Detection of *Pseudasphondylia matatabi* in *A. polygama* fruit galls**

316 Forty milligrams of dried *A. polygama* fruit galls were powdered by grating, and subsequently used to
317 isolate total DNA with Zymo's Quick-DNA Microprep Plus kit (#D4074) as per manufacturer's instructions.
318 Potential PCR inhibitors were removed using Zymo's OneStep PCR Inhibitor Removal kit (#D6030). *P.*
319 *matatabi* mitochondrial cytochrome c oxidase subunit 1 (COX) (GenBank AB085873.1) DNA was
320 amplified with AccuStart II PCR SuperMix and *P. matatabi* specific primers 5'–
321 AGGAACCTGGAACAGGGATGAACA–3' and 5'–AAAATTGGGTCTCCACCTCCT–3' (250 nM final
322 concentration) using the following program: 3 min. at 95°C, 35 x (30 sec. at 95°C, 30 sec. at 60°C, 30
323 sec. at 72°C), and 2 min. at 72°C. The 330 bp *cox1* amplicon was Sanger sequenced and BLASTn was
324 used to identify the species.

325

326 **DNA barcoding of plants**

327 DNA was isolated from 40 – 100 mg fresh leaves (*Actinidia* species) or 20 – 80 mg wood chips (*Lonicera*
328 species) using Omega Bio-Tek's E.Z.N.A. Plant DNA kit (#D2411-00) as per manufacturer's instructions.
329 *matK* was amplified with AccuStart II PCR SuperMix (QuantaBio #89235-018) and primers 5'–
330 CGTACAGTACTTTGTGTTACGAG–3' and 5'–ACCCAGTCCATCTGGAAATCTGGTTC–3' (250 nM
331 final concentration) (Weihong, Dawei and Xinwei 2018) using the following program: 3 min. at 95°C, 40 x
332 (30 sec. at 95°C, 40 sec. at 60°C, 60 sec. at 72°C), and 5 min. at 72°C. *rbcL* was amplified with AccuStart

333 II and primers 5'-ATGTCACCACAAACAGAAAC-3' and 5'-TCGCATGTACCTGCAGTAGC-3' (Weihong
334 et al. 2018) using the following program: 1 min. at 94°C, 35 × (10 sec. at 94°C, 20 sec. at 60°C, 45 sec. at
335 70°C). The *psbA – trnH* intergenic spaces was amplified using primers 5'-
336 GTTATGCATGAACGTAATGCTC-3' and 5'-CGCGCATGGTGGATTCAACATCC-3' (Sun et al. 2011) as
337 described for *rbcL*. After DNA cleanup using NEB's Monarch PCR Cleanup kit (#T1030G) the amplicons
338 of approximately 450 – 800 bp were Sanger sequenced using the forward and reverse primer. T-Coffee
339 (Notredame, Higgins and Heringa 2000) was used to align the sequences and the consensus sequence
340 was used to identified the species using NIH's nucleotide BLAST.

341

342 **Tinctures**

343 Tinctures were made by adding five volumes (500 mL) of absolute ethanol (Fisher Scientific, #BP2818) to
344 approximately 100 mL volume of plant materials inside a glass bottle. This included: dried catnip leaves
345 (Frontiers; 10 grams), Tatarian honeysuckle sawdust (20 grams) and dried valerian roots (50 grams). The
346 bottles were closed with a screw cap and were stored at room temperature in the dark with daily mixing
347 for 18 months. The liquid fraction was collected into a glass spray bottle by aspiration using a Pipet-Aid,
348 without disturbing the plant material sediment. Two sprays of the tincture (about 200 µL) were applied to a
349 fabric (empty polyester sock with 2 – 3% spandex), one on each side. All three tinctures and a control
350 fabric were offered for 5 hours on one afternoon/evening in October of 2020. No other olfactory stimulant
351 was offered to the cats at least two weeks prior to testing these tinctures.

352

353 **Fragrances**

354 Fabrics (empty polyester sock with 2 – 3% spandex) containing either a fragrance (**Table 5**) or negative
355 control (absolute ethanol) were offered to the cats on 8 different days between early September 2020 and
356 mid-December 2020. No olfactory stimuli were offered at least five days prior to testing of the fragrances.
357 Fabrics sprayed once on each side were made available to the cats immediately, whereas fabrics
358 sprayed abundantly (about 10 sprays) were left to stand for 10 hours at room temperature prior to making
359 them available to the cats (**Table 6**). Each sample was available to the cats for 15 hours (7:00 – 22:00).

360

361 **Chemical analysis of cat-attracting compounds**

362 The methodology published previously (Bol) to extract and quantitate known cat-attracting compounds
363 was optimized as described in detail below. Plant materials not already in powdered form were frozen
364 with liquid nitrogen and powdered using a mortar and pestle. Powdered samples (circa 500 mg) were
365 accurately weighed into glass vials in triplicate and 50 µg of internal standard (50 µL of 1 mg/mL
366 benzofuranone in ethyl acetate, Sigma #124591) was added per 500 mg tissue (10 µg per 100 mg). This
367 standard was chosen over the previously used tridecyl acetate standard because benzofuranone was
368 found to be more stable over time and it does not elute in regions of the chromatogram where other
369 analytes in the samples elute (especially *A. polygama* samples). Instead of 100% dichloromethane, we
370 used 5% (v/v) methanol / 95% dichloromethane as extraction solvent, since we found through
371 optimization that it is more effective at extracting the maximum amount of compounds of interest in 2 days
372 versus up to 7 days using 100% dichloromethane. Five mL of extraction solvent was added, the vials
373 were sealed and the content was magnetically stirred at room temperature for two days. One mL aliquots
374 were filtered and subsequently analyzed by gas chromatography–mass spectrometry (GC-MS). Samples
375 were analyzed on an Agilent 6890 instrument with autosampler connected to a 5973 mass selective
376 detector. The samples were separated using a DB5-ms capillary column (50 m × 0.2 mm × 0.33 µm, J&W
377 Scientific) using a flow rate of 0.7 mL/minute of ultra-high purity helium. The column initial temperature
378 was 40°C, held for 1 minute, then increased to 130°C at 10°C per minute, then increased at 2°C per
379 minute until 200°C, then finally at 15°C per minute to 280°C. The inlet temperature and transfer line were
380 set at 250°C and the mass spectrometer was set to record between 40 to 250 amu.
381 All compounds measured were confirmed with external standards and quantified by calibrating the
382 instrument using the internal standard method using the Agilent Enhanced Chemstation software (version
383 D.01.02.16). The instrument was calibrated against standard concentrations ranging from 0.1 µg/mL to
384 100 µg/mL of the pure compounds relative to the internal standard benzofuranone (10 µg/mL).

385

386 **Statistics**

387 The number of samples in this study was too small to assume normality of the data and therefore we
388 used non-parametric tests for the statistical analyses, with the exception of paired analysis with missing

389 data. The name of the test used for an analysis is mentioned in the text, immediately after a P value is
390 reported. Dunn's post-hoc test was always used for pairwise comparisons after the Kruskal-Wallis and
391 Friedman test. All P values reported from Dunn's post-hoc test are corrected for multiple comparisons. All
392 analyses were done using GraphPad Prism version 9. Color schemes were selected using ColorBrewer
393 (v2.0).

394 **Results**

395

396 **The duration of the response to cat-attracting plants differs between cats**

397 In a previous study, we tested the response of 100 domestic cats to *N. cataria*, *A. polygama*, *L. tatarica*
398 and *V. officinalis* (Bol et al. 2017). Results from that study indicated that cats who did not respond to *N.*
399 *cataria* (catnip) often responded to at least one of the other three plants. Because plants were available to
400 the cats for up to only one hour, we limited our analysis to scoring the absence or presence of the “catnip
401 response” and did not study their behavior in detail. Here we studied the response of 6 cats in their
402 familiar permanent home environment to the same 4 plants used in our previous study, plus *Acalypha*
403 *indica* (Indian nettle) (**Supplementary Figure 1B-G**), which has not been tested before to our knowledge.
404 To allow for a comprehensive analysis of cat behavior in response to the cat-attracting plants, each plant
405 was presented to the cats for a total of 100 hours, spread over 10 days (**Supplementary Figure 2A**). This
406 dataset was analyzed for differences in (1) response duration and (2) behavior in response to these
407 plants between (A) the cats and (B) the plants tested.

408 All but one of the 6 cats responded to all 5 plants tested (**Figure 1** and **Supplementary Figure 3**) and all
409 responses to the plants could be classified as “catnip responses”, meaning the cats showed (a
410 combination of) behaviors listed in Table 4. We observed approximately 2 hours of responses to *A.*
411 *polygama* and *L. tatarica*, 1.5 hours to *N. cataria* and *A. indica*, and 1 hour to *V. officinalis*. Since 5 of the
412 6 cats in this study had never responded to *N. cataria* in the past, two different brands of catnip were
413 used to investigate whether fluctuations in the level of active compounds in different sources of catnip
414 could account for variation in (or lack of) attractiveness. One sock contained catnip from the brand
415 Frontier, the other from the brand SmartyKat. When comparing the daily total response duration to both
416 catnip brands for each cat separately, we observed that cat O responded significantly longer to the catnip
417 from Frontier (**Supplementary Figure 4**). This finding suggests there may be a difference between the
418 two brands of catnip that were used in this study, but overall, many and robust responses were observed
419 from all 6 cats to catnip from both brands.

420 While previous work had suggested domestic cats respond euphorically to *A. indica* (Indian nettle) root in
421 a similar fashion to catnip (Scaffidi et al. 2016), this plant has never been tested on cats in a controlled
422 study. Since the cat-attracting effect of *A. indica* root quickly disappears after harvest (Scaffidi et al. 2016)
423 and its geographical distribution does not extend to North America, roots were lyophilized immediately
424 after collection on Christmas Island, Australia, in an attempt to preserve their effect on cats. Our data
425 show that the response duration to the lyophilized roots of Indian nettle was similar to the other plants that
426 were tested.

427 The cats only sparsely interacted with the negative controls (green tea). The total response time (any
428 engagement with the object, not behavior specific to the “catnip response”) from all cats to the negative
429 controls after 500 hours availability was just over 6 minutes, which is approximately 1% of the observed
430 response time to the cat-attracting plant materials (490 minutes). Nearly all interactions with the negative
431 control were from cat V and most of them occurred when *A. polygama* was tested. Three cats never
432 engaged with the negative controls.

433 There was no statistically significant difference in total response time of the cats between the 5 plants
434 (**Figure 1A**). Total response time is the sum of the duration of all responses, and is determined by both
435 response frequency and response duration. We also did not find a statistically significant difference in the
436 median response duration and response frequency of the cats between the cat-attracting plants.

437 However, when comparing the response duration to the 5 different plants between the 6 cats, we found
438 these to be significantly different (**Figure 1B**). Cats O and N responded longer to the cat-attracting plants
439 than cat Z. The differences in total response time to the cat-attracting plants between the cats could be
440 explained by both differences in the length of the responses and the frequency of responses. These data
441 show there are significant differences between cats in how long and frequently they respond to cat-
442 attracting plants.

443 There was no statistically significant difference in response duration between the various plants, possibly
444 because of the large variation between the cats. However, when we looked at the responses to the
445 various plants for each cat individually, we observed that cat H responded significantly longer to *A.*
446 *polygama* and cat O to *L. tatarica* and *N. cataria* than to some of the other plants (**Figure 2**). Interestingly,
447 cat Z showed no interaction at all with the sock containing *V. officinalis* root over the full 5-week testing

448 period.

449 The data also show that *N. cataria* (catnip) was not more popular than the other plants tested when
450 comparing across the 6 domestic cats in this study. The longest total response duration after 100 hours,
451 as well as the longest total response per day, and the longest single response was never to *N. cataria*
452 (**Supplementary Figure 5**). These results suggest that while catnip might be the best-known cat-
453 attracting plant among cat caregivers outside of East Asia, the other plants seem to be at least as potent.
454 Behavior observed for cats O and V in response to the plant *Menyanthes trifoliata* (buckbean) suggests
455 this plant is also able to elicit the “catnip response”. Fifteen grams of dried buckbean leaves (Siberian
456 Herbals) inside a sock was offered to cats A, N, O, V and Z for a couple of hours on one day. We
457 observed one response of cat O that lasted about half a minute and one response of cat V that lasted a
458 little over one minute.

459

460 **The degree of attraction to cat-attracting plants differs between cats**

461 Next, we looked at the degree of attractiveness of the plants. This was measured by the time it took a cat
462 to respond to the plant for the first time after it was made available on each of the 10 test days. The data
463 show no difference in attractiveness between the 5 plants we tested (**Figure 3A**). However, we did
464 observe significant differences in how strongly individual cats were attracted to the plants (**Figure 3B**).
465 These results suggest that the time to first response is in part determined by the cat's personality (e.g.,
466 curiosity or fear of missing out), rather than intrinsic properties of the plant. Therefore, we also compared
467 the times to first response to the 5 cat-attracting plants for each cat separately. Seeing differences in time
468 to first response between the plants for individual cats may suggest differences in intrinsic properties
469 between the plants. Similar to response duration, while we did not see differences between the time to
470 first response when we looked at the combined data of all 6 cats, we did see statistically significant
471 differences in time to first response between plants when we analyzed the data for each cat separately
472 (**Figure 4**). While cat O did not have a single day out of the 50 without responding at least once, cat Z did
473 not respond at all on about 70% of the days, including the 10 days *V. officinalis* was available. Cat O
474 responded to *L. tatarica* and *N. cataria* almost immediately on each of the 10 test days. In contrast, the
475 first response to *V. officinalis* of cat O was about 9 hours on three of the 10 test days. The opposite was

476 seen for cat V, who appeared to be attracted more strongly to *V. officinalis* than to *N. cataria*. On all 9
477 days that cat V responded to *V. officinalis*, this was within or around half an hour. These results suggest
478 that the level of attractiveness of a plant is not solely determined by properties of the plant, but also by
479 how the cat perceives the plant.

480 Taken together, these data show that all 5 plants are equally capable of attracting domestic cats and
481 eliciting the “catnip response”, while both response duration and how strongly individual cats are attracted
482 to the plants can differ significantly. These differences might in part be due to variation in olfactory
483 perception and in part to differences in the cats’ personalities.

484

485 **The “catnip response” is different between cats, but comparable among various 486 cat-attracting plants**

487 In addition to the quantitative analysis (i.e., duration of the response) we also studied the qualitative
488 aspects of the responses to the various plants. We created an ethogram that is specific for the “catnip
489 response” (**Table 4**). Some of these behaviors may be affected by how the olfactory stimulus is offered to
490 the cat. For example, biting and pulling with the object in the cat’s mouth will be possible when the plant
491 material or single compound is offered inside or on a fabric, respectively, but it will not be observed when
492 powder of dried *A. polygama* fruit galls is sprinkled on the floor. In this study, all plant materials and single
493 compounds were offered on or in a fabric and therefore allowed for comparison between cats, as well as
494 between plants or single compounds. Behaviors not mentioned and described in the ethogram either did
495 not occur (Flehmen, lordosis, vocalization) or were not analyzed because of limitations such as camera
496 angle and distance (e.g., drooling). Sniffing was not included because it was considered behavior used to
497 detect or identify an odor, not behavior in response to smelling odorants. Although not specifically studied
498 or analyzed, no signs of stress, fear or aggression (determined by for example positioning of the ears or
499 tail) were ever observed. In addition to previously described behavior in response to catnip, we have
500 added “rippling of the back” and “twitching of the back” (**Supplementary File 1**). This behavior is not
501 linked to feline hyperesthesia syndrome. There is no reaction (biting, scratching or licking of the area
502 where the twitching or rippling occurs) of the cats to the concerning area of the back, rather, the cat
503 seems completely unaffected by it. Twitching and rippling of the back appeared to be quite specific for the

504 “catnip response” since it was only rarely observed on other occasions. “Rolling on the side” reflects the
505 frequency of changes in body position (standing/sitting to lying on the side or lying on the side to lying on
506 the back). Rippling and twitching of the back, as well as rolling on the side and head shaking are
507 extremely short events and are therefore reported and shown as events per minute response, whereas all
508 other behaviors are reported and shown as the percentage of the total response time. The percentages
509 can exceed 100% since some behaviors can be displayed by the cats simultaneously (e.g., holding and
510 rubbing, or, holding and raking).

511 To compare behavior between the cats, we analyzed 5 responses to *N. cataria* nearest to 60 seconds of
512 each cat using BORIS behavioral analysis software. Catnip was chosen because the variation in
513 frequency and length of the responses of the 6 cats was least for this plant. During the response, the cats
514 were mostly either sitting or lying on their side. Time spent while standing or lying on their back during the
515 response was also observed, but not frequently (**Figure 5**). Body position during the response varied
516 enormously between the cats. Cat O predominantly lay on his side while engaging with the filled sock,
517 cats A, H and Z responded predominantly in a sitting position, and cats N and V showed an equal mix of
518 sitting and lying on their side (**Figure 6**).

519 Our data also suggest there is large variation between cats in most behaviors that are typical for the
520 “catnip response”. Head rubbing the olfactory object was the behavior observed most frequently, and
521 although it was seen for all 6 cats, there were significant differences between the cats (**Figure 6**,
522 **Supplementary File 4**). The response to *N. cataria* for cats A and H consisted almost exclusively of head
523 rubbing, significantly more than for cat O. In addition to head rubbing, cat O showed other behaviors such
524 as raking or biting while holding the object. The amount of time spent holding the sock, raking and biting
525 was significantly greater for cat O than for several of the other cats (**Figure 6**). Rippling of the back was
526 not seen for cats A and H but was a characteristic feature of cat Z’s response, where it was seen at high
527 frequency (**Figure 6**). In fact, about 15% of her response time was rippling of the back. Head shaking,
528 rolling on the side, and twitching of the back was seen for most or all cats, with no differences between
529 cats for the latter. The frequency of head shaking was significantly different between the cats O and Z
530 (**Figure 6**). This behavior seemed to be rather specific for the “catnip response” since it was not seen
531 during their normal daily activity. None of the cats had medical problems with their ears, nor did we

532 observe any buildup of wax in their ear canal to account for head shaking. We also did not see any
533 scratching or pawing aimed at the head or ears, which would be indicative of medical problems with the
534 ears. Perhaps this head shaking behavior is similar to “shake-off” behavior seen in dogs where it can
535 serve as a “reset button” after excitement, although there is no literature that would support this
536 hypothesis. Alternatively, it might be a way for the cats to shed excess saliva, since it is known that these
537 cat-attracting plants can induce drooling (Bol et al. 2017).

538 Overall, the frequency of rolling on the side was low. The responses of cats N and O seemed more
539 dynamic than the response of cats A and Z since rolling on the side from a sternal position, or onto the
540 back from a lateral position, was seen more frequently with cats N and O (**Figure 6**).

541 Collectively, these data demonstrate that the behavior seen in the “catnip response” is quite consistent for
542 each cat, but show enormous variability between cats.

543

544 Having observed large variation in response traits of domestic cats towards catnip, we wondered if their
545 idiosyncratic behavioral pattern would be the same for all the various cat-attracting plants used in this
546 study. As can be seen in **Figure 5**, the behavioral pattern in response to *N. cataria* is quite distinct
547 between cats A, O and Z. Cats A and Z have a fairly simple behavioral response where they
548 predominantly sat and head rubbed the object, with cat Z also frequently demonstrating rippling of her
549 back. On the contrary, cat O spent much more time lying on his side, raking, biting, and holding the
550 object, and rolled on his side much more frequently than the other two cats. To test if there is a difference
551 in behavioral patterns of cats towards different cat-attracting plants, we analyzed the behavior of cats A,
552 O and Z in response to all plants tested in this study.

553 During the response of cat A to any of the 5 plants, she predominantly sat and head rubbed the filled
554 socks (**Figure 7A**). While some licking was seen during some of her response to *A. polygama* and *V.*
555 *officinalis*, the body position and behaviors of cat A were highly similar between catnip and the 4 other
556 plants.

557 We observed lots of rippling of the back for cat Z in response to *N. cataria*. Behavioral analysis revealed
558 that rippling of the back was not specific for catnip, but rather part of her general response since it was
559 observed in response to all cat-attracting plants (except *V. officinalis* to which she never responded)

560 (Figure 7B). In addition to rippling of the back, we also observed twitching of the back in response to all
561 the other plants tested. It is unknown whether rippling of the back (wavelike motion) and twitching of the
562 back (single contraction on one location lasting a fraction of a second) are related. Her body position and
563 behavior during the responses to the other cat-attracting plants were highly similar in proportion and
564 frequency when compared to catnip.

565 Finally, we compared the behaviors of cat O between the 5 different plants. His response to *N. cataria*
566 was the most diverse and complex out of all the 6 cats with him predominantly in a lateral position (~85%
567 of the response time) when head rubbing (~50%), raking (~35%) and biting occasionally (~15%) while
568 holding the object (~50%). Cat O rolled on his side from a sternal position 2 – 3 times per minute
569 response duration, and we rarely observed headshaking (without the sock in his mouth), and rippling or
570 twitching of his back. In line with what we observed for cats A and Z, his behavioral pattern was near
571 identical for all cat-attracting plants (Figure 7C). The data also suggest however, that holding and raking
572 was seen less frequently for cat O when responding to *V. officinalis*, especially when compared to *N.*
573 *cataria* (Figure 7C and Supplementary Figure 6). These findings are interesting when considering the
574 previous observations that cat O was significantly less attracted to *V. officinalis* root than to *N. cataria*
575 (Figure 4), and that his total response duration to valerian root was also less than to other cat-attracting
576 plants (Figure 2).

577 Taken together, these data suggest that while responses between cats vary, the behavior of individual
578 domestic cats to diverse cat-attracting plants is highly similar, although the effect of *V. officinalis* root on
579 cats seems to be slightly different.

580

581 **Response duration to cat-attracting plants decreases with repeated exposure**

582 The setup of the experiments, with its repeated presentation, allowed us to learn more about possible
583 habituation (reduced response duration over time to the same stimulus) to the cat-attracting plants.
584 Information about possible habituation will be useful when giving advice to cat caregivers on how to use
585 olfactory stimuli for environmental enrichment. Furthermore, seeing differences in habituation between
586 plants might suggest the presence of different compounds or quantities of these compounds in the cat-
587 attracting plants.

588 The olfactory stimuli were offered 2 – 3 days a week, for 10 hours a day, for two periods of two weeks
589 (weeks 1 – 2 and 4 – 5), with an interstimulus interval of at least 9 days between weeks 2 and 4
590 (**Supplementary Figure 2A**). First, we compared the total response time (median of 6 cats) during the
591 first two-week testing period (weeks 1 and 2) with the second two-week testing period (weeks 4 and 5).
592 When we analyzed all 5 cat-attracting plants together, we found that the median response time was the
593 same (**Figure 8A**). We observed a similar pattern when we looked at the plants individually, suggesting
594 that either no habituation occurred within the 5-week testing period, or that the one-week interstimulus
595 interval was sufficient to reverse any habituation that may have occurred during the first two-week testing
596 period.
597 To test the latter, we compared the response duration between day 1 and day 5, as well as between day
598 6 and day 10. While none of the observed differences were statistically significant, we did see a decline in
599 response time to *A. polygama* within both the first and the second two-week testing period (**Figure 8B**).
600 The response duration on the last day of both 5-day testing periods (days 5 and 10) was shorter for
601 nearly all cats, suggesting that some habituation may have occurred. The response duration to this plant
602 was the highest of all plants tested on the first day of both 5-day testing periods.
603 To learn more about possible habituation to the various stimuli, we performed additional experiments
604 where the plant material was offered 10 days in a row for 2 or 12 hours per day. To rule out the effects of
605 potential degradation or complete volatilization of the active compounds over time, two new socks with
606 fresh plant material were offered every day. Habituation was observed for *A. polygama* (dried fruit gall
607 powder) and *L. tatarica* (sawdust) (**Figure 9**, days 1 – 10). A similar pattern was seen for *N. cataria* (dried,
608 cut leaves), but the difference between day 1 and day 10 was not statistically significant. We did not have
609 enough material to also test *A. indica*. For all plants tested, after 1 to 1.5 weeks of daily, voluntary
610 exposure (2 or 12 hours a day), the response duration of each cat was reduced to (close to) zero. After
611 the 10-day testing period and possible habituation to the plant materials, a different cat-attracting plant
612 was offered to learn if the scent from this stimulus would result in the reappearance of the response. This
613 dishabituation would suggest the presence of other active compounds or higher levels of similar
614 compounds in the newly offered stimulus. After habituation of the cats to either *L. tatarica*, *A. polygama* or
615 *N. cataria*, no dishabituation was seen when the cats were offered different cat-attracting plant material

616 (Figure 9). The only exception was cat O, who showed a longer response to *L. tatarica* than his first and
617 longest response to *A. polygama* and *N. cataria* (Figure 9A+D), underscoring the idiosyncrasy between
618 cats. Furthermore, these results suggest that *L. tatarica* may contain compounds not present, or at
619 significantly lower amounts, in catnip and silver vine. Another interesting finding was the observation that
620 offering *N. cataria* to the cats who were habituated to *A. polygama* and *L. tatarica* did not significantly
621 increase response duration. This might suggest that nepetalactone binds to (some of) the same olfactory
622 receptor(s) as some of the active compounds present in *A. polygama* and *L. tatarica*. These findings also
623 indicate that offering cat-attracting plants on a non-continual basis or alternating between the various cat-
624 attracting plants could prevent or reduce habituation in cats.

625

626 **Cat-attracting compounds in *A. polygama* are not exclusively produced in
627 response to the parasitic attack of the gall midge *P. matatabi***

628 Both normal *A. polygama* fruit and fruit galls used in our previous study (Bol et al. 2017) were collected
629 from vines growing in East Asia. In this natural habitat of the plant, gall midge *Pseudasphondylia matatabi*
630 females can lay their eggs in the plant's flower buds. As a result of this parasitic invasion fruit galls
631 develop. It seems that the presence of *P. matatabi* larvae in the developing kiwi fruit is critical for the
632 synthesis of compounds that serendipitously attract cats, since we have previously shown that domestic
633 cats respond to dried *A. polygama* fruit galls, but not to dried normal fruit (Bol et al. 2017). Indeed, we
634 were able to detect *P. matatabi* DNA in dried fruit galls that we used in our preceding study (Figure 10A).
635 Sequencing results confirmed, unequivocally, that *P. matatabi* DNA was present in the *A. polygama* fruit
636 galls (100% percent identity and query coverage; **Supplementary File 2**).

637 We wondered if the gall midge induces the synthesis of these compounds only locally (fruit) or
638 systemically (stem, leaves, fruit). It is known that some domestic cats do respond to dried *A. polygama*
639 stem (Bol et al. 2017). However, we do not know if these tissues were obtained from silver vine plants in
640 East Asia that were bearing fruit galls at the time of harvest. Since *A. polygama* is dioecious and *P.*
641 *matatabi* females deposit their eggs in the flower buds, not the fruit, one could argue that in response to
642 oviposition in a male flower bud the plant might also systemically induce synthesis of cat-attracting
643 compounds. However, *P. matatabi* oviposition in male flower buds or male flower bud galls have never

644 been observed (Dr. Junichi Yukawa, Kyushu University, Fukuoka, Japan, personal communication, June
645 2021). To test whether the presence of the gall midge is required for the synthesis of the cat-attracting
646 compounds, we grew *A. polygama* locally (Mico, Texas, USA), where *P. matatabi* does not occur. The
647 cats were offered dried leaves from the female Hot Pepper variety and the male Pavel variety, each for
648 almost a full day. Seeing cats respond to leaves from male plants, even when grown in their natural
649 habitat and hence in the presence of *P. matatabi*, would suggest that the gall midge is not required for the
650 production of these compounds. All five cats responded to the locally grown *A. polygama* leaves, both
651 from the male and female plant (**Figure 10B**). Although the data are limited, they strongly suggest the
652 leaves were at least as popular among the domestic cats as the dried gall material from East Asia. The
653 shorter response to the leaves from the Pavel variety may be explained by harvesting later or the longer
654 drying time of the leaves. Harvest time for those leaves was later in the fall when the leaves would soon
655 be shed by the plant. Testing these already collected leaves was postponed because we wanted cat A,
656 who had recently received radioactive iodine treatment for hyperthyroidism, to also participate. Stem from
657 the female silver vine Hot Pepper variety was made available to the cats on two different days. In
658 agreement with our previous findings (Bol et al. 2017), only a small percentage (20%) of the cats
659 responded to the silver vine stem. Cat Z responded 4 times: 26, 8, 18 and 22 seconds, with a total
660 response time of 74 seconds, and analysis of her behavior showed that the response was similar to the
661 behavior observed when exposed to the other cat-attracting plants: mostly head rubbing in a sitting
662 position with her back rippling and an occasional head shake (**Figure 10C**). No responses were seen to
663 the control stem (lignified *Juniperus ashei*). Interestingly, while cat Z responded for a total time of 4
664 minutes and 15 seconds to the dried leaves of the Hot Pepper variety, she did not touch the sock
665 containing the leaves for approximately half of that time. No other responses where there was no contact
666 with the test object by cat Z or any other cat to any plant material were seen. Instead of contact with the
667 object, she rubbed her head on the floor, rolled on her side, and her back rippled, all in close proximity
668 (approximately 20 cm) to the olfactory object. This observed behavior in response to the dried silver vine
669 leaves was characteristic for her and highly similar to her responses to other plants. This cat never
670 demonstrated this behavior in response to any of the controls, which were available for hundreds of
671 hours, and her most recent response prior to these responses was 3.5 weeks earlier. Therefore, we

672 concluded this response was specific to the *A. polygama* leaves.

673 We previously concluded that domestic cats do not respond to *A. polygama* leaves grown in the USA (Bol
674 et al. 2017). However, subsequent DNA barcoding (*matK*) revealed that the leaves previously used for
675 testing were from the closely related species *Actinidia arguta* instead of *Actinidia polygama*. These *A.*
676 *arguta* leaves were only used for one small experiment in our previous study, and this finding does not
677 change any of the main or other conclusions of the published work. DNA barcoding (*matK*, *rbcL* and *psbA*
678 – *trnH*) results strongly suggest we have used *A. polygama* for all experiments in this study, although we
679 could not rule out the closely related *A. valvata*. Since the use of Tatarian honeysuckle wood as olfactory
680 enrichment for cats is still uncommon and, as far as we know, is only available from one source (The Cat
681 House in Calgary, Alberta, Canada), we also used DNA barcoding (*matK*, *rbcL* and *psbA* – *trnH*) to
682 confirm that what we used in this study was indeed *Lonicera tatarica*. All sequences can be found in
683

Supplementary File 2.

684 In conclusion, these findings show that while the gall midge *P. matatabi* seems to induce a change in the
685 plant's volatile pattern in the kiwi fruit gall, oviposition in the flower buds does not seem to be required to
686 develop the cat-attracting characteristics of the stem and leaf tissues in either male or female silver vine
687 plants.

688

689 Active compounds in plants can be extracted using ethanol

690 We created *N. cataria*, *L. tatarica* and *V. officinalis* tinctures to determine whether this easy extraction
691 method would result in a product that could attract and stimulate domestic cats. A liquid (ethanol) form
692 would offer several possible advantages over the plant form since it can be applied to any object. *A.*
693 *indica* and *A. polygama* tinctures were not created because of limited availability of plant material. We
694 were also curious to see if we could extract any active compounds of dried *V. officinalis* root with absolute
695 ethanol, and possibly avoid co-extracting any compounds that may have had an inhibitory effect on cat Z.
696 She was the only cat who did not respond to *V. officinalis*, despite the plant being available for 10 days,
697 10 hours a day. We hypothesized that cat Z did not respond because dried *V. officinalis* roots have, at
698 least to most humans, a strong, unpleasant or repulsive smell.
699 We applied two sprays of the tincture and two sprays of ethanol only (negative control) on a piece of

700 fabric which were subsequently made available to five cats for a total of 5 hours in the afternoon /
701 evening. We observed positive responses of two to four cats to each tincture (**Figure 11**), and the
702 responses to them matched the “catnip response” behavior that was characteristic for each cat.
703 Interestingly, despite the characteristic valerian root smell still being present, cat Z did respond to the *V.*
704 *officinalis* root tincture, and this single response of nearly one and a half minutes was longer than 90% of
705 all her responses to the plants tested. Furthermore, while cat Z also responded to the catnip and Tatarian
706 honeysuckle tinctures, her response to the valerian root tincture was the longest. Although we only
707 applied two sprays of each tincture, we still observed responses of all 5 cats 3.5 hours after we
708 application (cats A and N to the *V. officinalis* tincture, cats O, V and Z to the *L. tatarica* tincture, and cat Z
709 to the *N. cataria* tincture).

710 The results from this experiment suggest that at least some of the active compounds found in the cat-
711 attracting plants can be effectively extracted simply by soaking the plant materials in absolute ethanol.
712 Although cat Z did not respond to dried valerian root, she did respond to the tincture, suggesting
713 compounds responsible for inhibiting her attraction were not coextracted with the active compounds.
714 However, it is also possible that she preferred different amounts or ratios of the compounds in the
715 tincture.

716

717 **Domestic cats respond to all iridoids, including dihydroactinidiolide, but**
718 **response to actinidine is rare**

719 We have previously shown that cats respond to cat-attracting plants known to contain little to no
720 nepetalactone (Bol et al. 2017). While we detected iridomyrmecin, isodihydronepetalactone and actinidine
721 in these plants, we did not confirm whether these compounds are responsible for the cat-attracting
722 properties of these plants. The main goal of this experiment was to determine to which compounds
723 identified in cat-attracting plants domestic cats would respond. Furthermore, we were interested to see if
724 the differences in response between various cats to the individual cat-attracting plants (e.g., cat O
725 responding significantly longer to *L. tatarica* than cat Z) and the differences in response of individual cats
726 to the various plants (e.g. cat O responding significantly longer to *L. tatarica* than to *V. officinalis*) could be
727 explained by different responses to the single compounds.

728 In these bioassays, performed with the same cats who also tested the plant materials, we tested not only
729 the lactones *cis-trans*-nepetalactone (**1**), *trans-cis*-nepetalactone (**2**), isodihydronepetalactone (**4**),
730 iridomyrmecin (**7**) and actinidine (**9**), but extended the repertoire by adding the lactones
731 dihydronepetalactone (**3**), neonepetalactone (**5**), isoneonepetalactone (**6**), isoiridomyrmecin (**8**), the
732 pyridine actinidine (**9**), the furanone dihydroactinidiolide (**10**), and indole (**11**) (**Figure 12, Table 3**). This
733 selection (compounds **1 – 10**) was based on previous reports in the literature and summarized in the
734 review by Arthur and Sharon Tucker (Tucker and Tucker 1988). We attempted to obtain or synthesize
735 several other compounds mentioned in the work of Tucker and Tucker, such as boschniakine, but they
736 were either not commercially available or unstable. In our hands, boschniakine was found to be
737 particularly unstable when prepared through chemical synthesis. One hypothesis as to why cats respond
738 to these molecules is that they resemble cat pheromones found in cat urine, feces, and glandular
739 secretions. We identified indole as the only known compound in feline excretions that showed structural
740 resemblance to the known cat-attracting compounds (Starkenmann et al. 2015, Miyazaki et al. 2018,
741 Uetake et al. 2018) and therefore we also tested this compound as a cat-attractant. Thirty-three, 100, 300
742 and 900 µg of each compound was made available to the cats on two different days, for a total of at least
743 17 hours per compound.

744 We found that all of the plant-derived compounds (**1 – 10**) elicited a positive response in domestic cats,
745 but not the negative control (evaporated diethyl ether) nor indole (**Figure 13A, Supplementary Figure 7**).
746 All responses could be classified as “catnip responses”. There was no statistically significant difference in
747 median response duration of the 5 cats between the active compounds ($P > 0.05$, Friedman test). The
748 response time among cats to actinidine had a larger range and more uneven distribution than any of the
749 other compounds (**Figure 13A**). Three out of the 5 cats showed no or little interest in this compound.
750 Therefore, we tested actinidine on three additional days. All actinidine data shown is from 5 days of
751 testing, between January and May 2019, totaling 53 hours of exposure (**Supplementary Figure 7**). We
752 also made fabric with a higher amount of actinidine (2700 µg) available for 4 hours to compensate for
753 potential variation between the cats in their detection threshold for this single compound. The three cats
754 who did not respond to actinidine were cats O, N and V. Interestingly, these cats had the longest total
755 response time to all other compounds (**Figure 13B**). While most cats did not respond to actinidine, cat A

756 responded longer to actinidine than to any of the other compounds that were tested (**Figure 13B**). The
757 response duration of cat A to actinidine was 5 – 9 × longer than her response to the lactones. These data
758 did not provide information on how common the response to actinidine is among domestic cats, especially
759 since the three non-responders are suspected to be genetically related. However, recently published
760 supplementary data by Reiko Uenoyama and her colleagues that was not analyzed or discussed in their
761 article (Uenoyama et al. 2021) strongly suggest that a response to actinidine is less common given only
762 one of 12 cats in their study responded to actinidine (**Figure 13C**). Furthermore, all 11 other cats who did
763 not respond to actinidine responded to most (approximately 5 out of 6) of the lactones that were also
764 tested (**Figure 13C**). These results from the study of Uenoyama *et al.* reinforce our findings. Uenoyama *et*
765 *al.* tested 50 µg of the single compounds. Since we observed more than half of the total response time of
766 cat A to actinidine when we used 33 and 100 µg, it is unlikely that the absence of a response of those 11
767 cats would be due to the amount of actinidine used in their experiments.
768 The longer response time of cat A to actinidine compared to the lactones could be explained by both an
769 increased response frequency and duration of the individual responses. Cat A responded to actinidine
770 once every 1.75 hours, compared to roughly once every 6 hours for the lactones, which is almost 3.5 ×
771 more frequent. The median response duration to actinidine of cat A was statistically significantly longer
772 than to the lactones (42 and 18 seconds, respectively; **Supplementary Figure 8**).
773
774 For the analyses described above, data were pooled from tests with various quantities of the single
775 compounds (33, 100, 300, 900 and for actinidine even 2700 µg) performed on different times of the day
776 (morning, afternoon, evening). We used data from the compounds for which we observed at least 10
777 responses of an individual cat to look for possible correlation between quantity of the compound and
778 response duration/frequency. Cat A responded 30 times to actinidine (**9**), and cat O responded 14, 10
779 and 10 times to compounds (**2**), (**3**) and (**4**), respectively. The data show absence of a dose-response
780 relationship at quantities ranging from 33 to 2700 µg (**Supplementary Figure 9A-B**). Furthermore, we
781 found that the distribution of responses matched the distribution of the hours the olfactory test objects
782 were available to the cats through the day (**Supplementary Figure 9C**). This result indicates the cats
783 were not less active in the afternoon, which may have resulted in fewer responses during this part of the

784 day. Taken together, these data suggest that pooling data (different quantities and tests performed at
785 different times of the day) did not affect the results and conclusions.

786

787 When we compared the cats' response duration to the plants with the response duration to the single
788 compounds, we found a very strong positive correlation (**Figure 14**). The response duration to the cat-
789 attracting plants was approximately 33% longer than to the single compounds. This might be explained by
790 higher quantities of compounds in the plants, the presence of multiple compounds, slower and more
791 sustained release of compounds, larger volume of the test object, or a combination of these.

792

793 **The degree of attraction to the single compounds differs between cats**

794 Similar to what we observed for the plants (**Figure 3B**), we found that the time to first response to the
795 single compounds was significantly different between cats (**Figure 15**). When we looked at the data for
796 each cat separately, we also found significant differences in time to first response between the different
797 classes of single compounds (lactones, actinidine, dihydroactinidiolide). As expected, cat A was
798 significantly more attracted to actinidine than to the lactones or dihydroactinidiolide, whereas the opposite
799 was seen for cat V. The time to first response to actinidine of Cats N and O was also longer compared to
800 the lactones, but the difference was not statistically significant because of an outlier. The responses of
801 cats N (n=1) and O (n=2) to actinidine lasted only a few seconds and might be considered 'false
802 positives' (see below).

803 These findings support the previous observation that there is variation between cats in how attracted they
804 are to certain cat-attracting scents. These data also strengthen the hypothesis that actinidine is distinct,
805 not only in structure, but also in the effect it elicits in domestic cats. The near immediate (seconds after it
806 was made available) "response" from cat O to actinidine supports the hypothesis that the time to first
807 response is at least in part determined by the cat's personality (i.e., curiosity, fear of missing out).

808

809 **Behavioral response to actinidine is different from responses to lactones and cat-
810 attracting plants**

811 Next, we analyzed the behavior of cat A using BORIS software to determine if there was a difference in
812 her behavior when exposed to plants, lactones and actinidine. Since the responses of cat A to the various
813 plants (n=5) was highly similar (**Figure 7A**), we only used the *N. cataria* data for the comparison to the
814 single compounds. For the plants, five responses nearest to one minute were analyzed. To keep the
815 median response time similar, we only analyzed responses of cat A to the lactones and actinidine with a
816 duration between 30 – 90 seconds (n=9 and n=16, respectively). Interestingly, cat A spent significantly
817 more time licking the object with actinidine and less time head rubbing, when compared to the responses
818 to the lactones or *N. cataria* (**Figure 16** and **Figure 17**). The same statistically significant differences were
819 seen when all responses to actinidine and the lactones longer than 30 seconds were analyzed (n=11 and
820 n=24, respectively), capturing 95% and 83% of the total response duration to these compounds,
821 respectively. The percentage head rubbing was lower for actinidine as the result of more time spent
822 licking. Other than a difference in the frequency of head shaking, no differences were seen in any of the
823 other behaviors.

824 It seems that the observed licking of cat A is a true feature of her response to actinidine and not the result
825 of longer response durations that we have seen for actinidine compared to the lactones (**Supplementary**
826 **Figure 8**). Indeed, we found no correlation between the percentage of response time licking and
827 response duration (**Supplementary Figure 10A**). Although licking was the dominant behavior observed
828 for the two responses to the fabric with the highest amount of actinidine (2700 µg), the correlation
829 between the amount of actinidine and the percentage of response time spent licking was weak
830 (**Supplementary Figure 10B**).

831
832 Cat Z also responded to actinidine, but the responses were much less frequent and shorter in duration
833 compared to cat A. Three short responses (10 – 20 seconds) and one response of almost one minute
834 were observed. While active engagement (contact) with the object was a requirement for any feline
835 activity to be considered a response, about 90% of the time that cat Z responded to actinidine she did not
836 touch the object. This lack of contact during the response was also seen for freshly harvested, locally-
837 grown *A. polygama* leaves, plant material known to contain relatively large amounts of actinidine (Bol et
838 al. 2017). However, the response occurred in close proximity to the test object and her behavior was

839 characteristic of what was seen with the other plants and compounds: head rubbing (the floor near the
840 object) in a sitting position, rippling and twitching of her back and occasionally rolling on her side. Only cat
841 Z demonstrated responses without touching the olfactory object. Since cat Z did not respond to any of the
842 negative controls that were available for hundreds of hours, and given her most recent response to any
843 olfactory stimulus prior to actinidine was 3 months earlier, we believe this response was specific.
844 The median response duration to the lactones (n=10) and actinidine (n=4) of cat Z was 26 and 15
845 seconds, respectively. Therefore, we included her two shorter responses to *N. cataria* in the qualitative
846 and statistical analysis. As a result, we compared all her responses to catnip (n=7), all responses to
847 actinidine and all responses < 60 seconds to the lactones. We also observed some differences between
848 her responses to catnip, the lactones, and actinidine. It appeared that the response of cat Z to actinidine
849 was more dynamic. Cat Z rolled on her side more frequently in response to actinidine than in response to
850 *N. cataria* or the lactones (**Figure 17** and **Supplementary Figure 11**). Rippling of the back was seen less
851 in response to the lactones as compared to catnip and actinidine, and for this reason the contribution of
852 head rubbing to the total response duration of the response increased. Head shaking was also seen less
853 frequently during responses to the lactones compared to catnip. When the 12 responses of cat Z to the
854 other plants (*A. indica*, *A. polygama* and *L. tatarica*) (**Figure 7B**) were included in the statistical analysis,
855 the results remained unaffected, except that the difference in the frequency of rippling of the back
856 between actinidine and the lactones also became statistically significant ($P < 0.05$; data not shown).
857

858 The response of cat O to actinidine was uncharacteristic for him and did not resemble the “catnip
859 response”. Both of his extremely short responses to actinidine (each about 10 seconds) lacked rubbing of
860 the object, which was seen in all his responses to the plants and lactones (**Figure 17**). While the behavior
861 of cat O to type I and II lactones (this discrimination is made based on the position of the carbonyl group;
862 see **Table 3** and **Figure 12**) was near identical, less holding and raking of the object was seen for the
863 lactones compared to the 15 grams of plant material (**Figure 17**), possibly due to lack of volume of the
864 object.
865

866 Collectively, these data suggest that while the responses to the single compounds are in general similar

867 to the behavior seen in response to the cat-attracting plants, there appear to be biologically significant
868 differences between actinidine and the lactones.

869

870 **Behavioral response to dihydroactinidiolide is similar to behavior in response to**
871 **lactones**

872 Another molecule that is structurally different from type I and II lactones (as well as actinidine) is
873 dihydroactinidiolide (**Figure 12**), which contains a furanone ring (5 membered lactone) compared to
874 pyranone rings (6 membered lactone). Interestingly, unlike the compounds **1 – 9** tested in this study that
875 have only been detected in plants or insects, dihydroactinidiolide has additionally been detected in
876 glandular secretions and urine of the red fox (McLean, Nichols and Davies 2021, Albone 1975, McLean,
877 Davies and Nichols 2017). None of the other iridoids tested here are produced or secreted by a mammal
878 to our knowledge. We wanted to determine if the behavior of cats triggered by this compound was similar
879 to the behavior seen in response to the cat-attracting plants and the other single compounds. Four out of
880 5 cats responded to this compound; however, the number and duration of the responses were low (13
881 responses in total for all 4 cats with a median response duration of 20 seconds) (**Figure 13A**). Of the cats
882 exposed to dihydroactinidiolide, cat V responded most frequently (n=5) and therefore the behavior she
883 demonstrated during those 5 interactions was analyzed using BORIS to compare to her behavior to *cis*-
884 *trans* nepetalactone and *N. cataria*. The behavior seen in response to *N. cataria* and nepetalactone –
885 sitting and head rubbing the object, holding the object while on her side, raking, and biting it, rolling on her
886 side, and shaking her head – was also observed for dihydroactinidiolide (**Figure 17** and **Figure 18**). Head
887 rubbing was again the dominant behavior, making up about 85% of the response time. There were no
888 significant differences in behavior between catnip, nepetalactone and dihydroactinidiolide.
889 Cats N and O both responded only twice to dihydroactinidiolide and therefore we did not perform
890 statistical analysis to test for differences. Responses of cat N to the plants were typically in a sternal or
891 lateral position and included mostly head rubbing (60 – 80% of response duration), sometimes while
892 holding the object. She also rolled on her side or back, about 2 – 3 times per minute of response duration
893 and rippling of the back was also seen, about 3 times per minute. When we compared this with her
894 behavior in response to dihydroactinidiolide, we noticed that head rubbing was still the most dominant

895 behavior (about 80% of the response time) with rippling of the back making up the majority of the balance
896 (about 2 times per minute). However, no holding of the object and no rolling on the side, and hence no
897 response in a lateral position, were observed (data not shown; **Supplementary File 3**).

898 The two responses of cat O to dihydroactinidiolide were short (10 and 20 seconds), but resembled his
899 responses to the plants: head rubbing, biting, holding the object, and raking were all seen, while in lateral
900 position (**Figure 17**).

901 The behavior of cat Z in response to dihydroactinidiolide matched her typical behavior when exposed to
902 plants and single compounds. She responded in a sitting position, head rubbing the object while her back
903 rippled. Since we observed only a single response from her, we could not test for statistical differences
904 between dihydroactinidiolide or other cat-attracting plants or compounds. However, the behavior of her 66
905 seconds response was near identical to the behavior seen during her responses to the lactones (**Figure**
906 **17**).

907 While the number of observations were limited and the duration of the responses was often short, we
908 believe these data show that the behavioral response to the structurally distinct dihydroactinidiolide is
909 highly comparable to the behavior seen in response to the other single compounds and the cat-attracting
910 plants.

911

912 **Stability of the single compounds**

913 We chose diethyl ether as solvent because of its inert nature and volatility, meaning it would evaporate
914 quickly and leave only pure compounds behind. The compounds were tested immediately after they were
915 dissolved in diethyl ether because information about their stability is lacking. Some of the *trans-cis*-
916 nepetalactone, neonepetalactone, isoneonepetalactone and actinidine dissolved in diethyl ether was
917 stored between experiments for a variety of reasons, as explained in detail in the “Materials and methods”
918 section. The results obtained with these compounds gave us some insight into the stability of these
919 compounds in diethyl ether under various conditions. When comparing the results between compounds
920 that were used immediately after dissolving in diethyl ether and those that were stored after dissolving,
921 we did not find any clear evidence of reduced activity, suggesting they were stable. When *trans-cis*-
922 nepetalactone was tested on two additional days, after being stored at room temperature for 1.5 months,

923 both the response frequency and total response time for all cats combined was higher on these days (C
924 and D) (18 responses, 17.6 minutes) compared to days A and B (9 responses, 10.5 minutes). We also did
925 not observe reduced response duration to neonepetalactone on day B after the dissolved compound had
926 been stored at 4°C for 4 days. 75% of the responses to neonepetalactone occurred on day B, whereas
927 this was 50 – 85% for the other lactones. While dissolved actinidine was stored for two weeks at various
928 temperatures ranging from freezing to room temperature, we still observed minutes of response to this
929 compound, albeit only during the first 4 hours of a 15-hour testing day (**Supplementary Figure 7**,
930 actinidine, day C). The absence of responses in the afternoon and evening were in contrast with what
931 was observed on days when actinidine was used immediately after dissolving in diethyl ether (days B and
932 E). Any possible degradation of actinidine would not affect the conclusions drawn in this manuscript since
933 this only would underestimate the true response of cat A.

934

935 **Any cat-attracting property of (pepper)mint is not caused by structural
936 resemblance of the active compound(s) to molecules like nepetalactone**

937 In addition to *Nepeta cataria*, there are several other plants from the genus *Nepeta* that contain cat-
938 attracting type I and II lactones (Formisano, Rigano and Senatore 2011, Regnier, Waller and Eisenbraun
939 1967, Bicchi, Mashaly and Sandra 1984, Eisenbraun et al. 1980), of which *Nepeta mussinii* or catmint is
940 arguably the best-known. Although all these plants are members of the Lamiaceae family, commonly
941 referred to as the mint family, plants in the *Nepeta* genus are not closely related to plants in the *Mentha*
942 genus, such as peppermint. There are numerous anecdotes of cats being attracted to peppermint
943 (*Mentha piperita*) and topical analgesics such as Bengay, IcyHot and Vicks VapoRub (which should all be
944 kept away from cats). Interestingly, *L. tatarica* (Tatarian honeysuckle) wood has a minty smell. Therefore,
945 we studied how domestic cats respond to the odiferous molecules menthol and methyl salicylate that are
946 responsible for the characteristic mint fragrance. Fabrics containing 33, 100, 300 and 900 µg menthol or
947 methyl salicylate were tested separately, and each was available on two different days for a total of 17
948 hours (5 hours on the first day, 12 hours on the second day). None of the cats responded to either of the
949 two compounds.

950

951 **Fragrances**

952 Anecdotal evidence from the past decade suggests that big cats (cheetahs and cats of the *Panthera*
953 genus: lion, tiger, leopard, snow leopard and jaguar) respond to certain fragrances (e.g. perfume, eau de
954 toilette), Calvin Klein's Obsession for Men in particular, in similar fashion to catnip (Banham Zoo, Norfolk,
955 England: Time 2020, BBC 2020 and The Washington Post 2020; Taronga Zoo, Sidney, Australia:
956 Scientific American 2014; Brookfield Zoo, Chicago, IL: CBS 2010; Bronx Zoo, New York, NY: Wall Street
957 Journal 2010 and National Geographic 2010). Patrick Thomas and his colleagues published the results of
958 a scent study in the Bronx Zoo where the responses of two adult cheetahs to 24 different fragrances were
959 studied (Thomas et al. 2005). The researchers applied three sprays of each fragrance to an object in their
960 1,000 m² outdoor naturalistic enclosure on three different days and reported the mean latency to inspect
961 the scent, the mean number of visits to the scent, the mean contact time to the scent and if head rubbing
962 was observed. All but one of the 24 fragrances were investigated by at least one of the two cheetahs,
963 demonstrating how scents can be used for environmental enrichment. However, head rubbing was only
964 seen in response to seven fragrances, and the median contact time to these was significantly higher than
965 the contact time to the other fragrances.

966 We were interested to see if the response of domestic cats to fragrances is similar to the response of big
967 cats. Furthermore, since many fragrances contain essential oils obtained from plants, we wondered if the
968 presence of compounds such as nepetalactone, iridomyrmecin, actinidine or dihydroactinidiolide in the
969 fragrances could be responsible for the observed behavior of the cats. It is known that lions, jaguars,
970 leopards, snow leopards and bobcats respond to plant material containing these compounds (*N. cataria*
971 or catnip and *A. polygama* or silver vine) (Todd 1963, Bol et al. 2017). If these compounds are present in
972 perfumes, colognes or eau de toilettes, then we would expect domestic cats who respond to plant
973 materials containing these stimulants to also be attracted to these fragrances. To test this hypothesis, we
974 selected the four most popular fragrances (head rubbing by both cheetahs and longest average contact
975 time: 668, 662, 207 and 185 seconds) of the 24 used in the study by Thomas and colleagues. We applied
976 them to a polyester fabric and made the fragrances available to domestic cats A, N, O, V and Z who all
977 responded to most or all of the tested plants and single compounds. The fabrics were sprayed twice

978 (approximately 200 µL) with either Obsession for Men, L'Air Du Temps, Paco Rabanne Pour Homme,
979 Drakkar Noir, or ethanol as a negative control (**Table 5** and **Table 6**). The fabrics were then made
980 available to the cats for 15 hours, from 7:00 till 22:00 upon which all cats investigated the fabrics several
981 times. Cat A responded to the Drakkar Noir for a duration of 3 minutes and 50 seconds (**Figure 19A**) and
982 this response resembled the behavior seen in her responses to the cat-attracting plants and single
983 compounds: head rubbing and licking of the fabric with the fragrance while in a sitting position, shaking of
984 the head and occasional twitching of the back (**Figure 19B**). None of the other cats responded to any of
985 the other three fragrances tested, including the popular (among big cats) Calvin Klein's Obsession for
986 Men. Therefore, we tested Obsession for Men a second time, one week later, again for 15 hours, and
987 used a bottle obtained from a different source. Drakkar Noir was also made available for a second time.
988 Again, only cat A responded to Drakkar Noir, this time for 3 minutes and 40 seconds (**Figure 19A**), while
989 none of the cats responded to the fabric with Obsession for Men. In both cases, the response of cat A to
990 Drakkar Noir occurred approximately 14 hours after the fabric was made available. After this amount of
991 time in a well ventilated, open area, it is expected that only larger, less volatile molecules will remain on
992 the fabric, such as nepetalactone, iridomyrmecin, actinidine and dihydroactinidiolide that are found in the
993 essential oils of cat-attracting plants. In addition to base notes (larger, less volatile molecules), fragrances
994 have what is referred to in the fragrance industry as top and middle (heart) notes consisting of molecules
995 that can be detected more quickly. Some of these smaller, more volatile compounds may interfere with
996 the detection or perception of other, potentially cat-attracting molecules. To increase the exposure time to
997 the larger single compounds only, fabrics sprayed about 10 times were left to stand overnight at room
998 temperature (at a location where the cats could not smell them) before making them available to the cats.
999 While cat O briefly (a few seconds) interacted with the fabric sprayed with Obsession for Men, his
1000 behavior was contrary to his responses to the plants and single compounds (sitting, no head rubbing and
1001 no raking). This alternative methodology did not lead to any other responses of the cats to the fragrances.
1002 To decrease the chance that the lack of response to Calvin Klein's Obsession for Men were false
1003 negative results, a third source of this fragrance was made available to the cats a fourth and fifth time, but
1004 no responses were observed.

1005 Many have speculated that the response of cats to Calvin Klein's Obsession for Men is due to the
1006 presence of the molecule civetone (Mandy Aftel, The Washington Post 2020 and NPR 2018; Miguel
1007 Ordeñana, Scientific American 2013; Ann Gottlieb, Wall Street Journal 2010). Civetone is an odiferous
1008 ketone found in civet, a glandular secretion of the civet cat (*Anonis* 1997) but it can also be synthesized
1009 (Tanabe 2002). To test if domestic cats would respond to civetone or civet we sprayed 0.1 and 1%
1010 civetone (a kind gift from Fred Keifer at Firmenich), a fragrance known to contain civetone (Civette
1011 Intense), and 1% absolute civet (a synthetic recreation of natural civet) on a fabric and made each
1012 available to the domestic cats for 15 hours (**Table 5** and **Table 6**). For ethical reasons, we decided not to
1013 obtain and test natural civet. None of the five cats showed any interest in the fabrics containing these
1014 scents (**Figure 19A**).

1015 These data suggest that domestic cats do not respond to fragrances like big cats do and that the
1016 response of big cats to fragrances such as Obsession for Men is unlikely triggered by the presence of
1017 compounds similar to nepetalactone, iridomyrmecin, actinidine or dihydroactinidiolide. Indeed, GC-MS
1018 analysis of Obsession for Men revealed that no cat-attracting single compounds were detected in this
1019 fragrance. The similar negative result for Drakkar Noir suggests the presence of (an) other, unidentified
1020 cat-attracting compound(s) in this fragrance.

1021 While domestic cats do not seem to respond to civetone, this conclusion does not exclude the possibility
1022 that the big cats do, since the fragrances that were highly popular among cheetahs were not very popular
1023 among the domestic cats.

1024

1025 **Cat-attracting plants contain a wide array of nepetalactone-like molecules**

1026 Previously, we quantified 5 cat-attracting molecules (nepetalactone, epinepetalactone,
1027 isodihydronepetalactone, iridomyrmecin and actinidine) in the plant materials that we used in our
1028 preceding study (Bol et al. 2017) using tridecyl acetate as an internal standard. Here, we were able to use
1029 the synthesized single compounds as standards, which were previously not available. Therefore, we were
1030 able to quantitate these compounds more accurately and quantitate additional compounds in the plant
1031 tissues. We again analyzed catnip leaves, silver vine fruit gall, Tatarian honeysuckle wood, and valerian
1032 root. We now also included Indian nettle root, Texas-grown silver vine leaves, and lignified silver vine

1033 stem (**Table 2**). All these plant tissues were from the same batches that were used in the experiments
1034 described in this article. We also performed GC-MS analysis on samples from inside the socks that were
1035 used for testing. We did not find any evidence that would suggest significant loss of active compounds as
1036 the result of interactions with the cats (e.g., contact with saliva) over the duration of the experiments
1037 (**Supplementary Figure 12**).

1038 As expected, nepetalactone and a 5 – 10-fold lower amount of epinepetalactone were only detected in
1039 the *Nepeta cataria* samples (**Figure 20**). In addition to these two compounds, nearly all other known cat-
1040 attracting compounds were detected in catnip, except for neonepetalactone and isoneonepetalactone.
1041 We found that one of the two brands of catnip contained large amounts of dihydronepetalactone and
1042 isodihydronepetalactone (which are reduced form of nepetalactone). However, surprisingly, this
1043 difference of about one log did not result in an increased response time of the cats (**Supplementary**
1044 **Figure 4**). In fact, the opposite was observed for cat O: a significantly longer response time was seen for
1045 Frontier catnip. *A. polygama* fruit galls also contained a large number of active compounds (n=9),
1046 including *trans*-dihydronepetalactone and *trans*-isodihydronepetalactone, which are reduced forms of
1047 epinepetalactone. The amount of actinidine in the silver vine fruit galls was about one log more than what
1048 was extracted from the catnip leaves. The fruit galls contained neonepetalactone and
1049 isoneonepetalactone, which were absent in catnip, but did not contain dihydroactinidiolide. The latter was
1050 only found in catnip. Surprisingly, the chemical composition of Tatarian honeysuckle wood was highly
1051 similar to that of valerian root and Indian nettle. These plant tissues contained relatively large amounts of
1052 actinidine in addition to two or three other compounds (isodihydronepetalactone, isoiridomyrmezin,
1053 iridomyrmezin).

1054
1055 GC-MS analysis was also done for *M. trifoliata* (buckbean) leaves, as well as other silver vine tissues
1056 (stem and leaves) and three tinctures that we used in our experiments (**Figure 20**). Only
1057 dihydroactinidiolide was detected in the *M. trifoliata* leaves. Of the two cats who responded to *M. trifoliata*
1058 it was Cat V who responded longest. She was also the one who responded the most frequent and the
1059 longest to dihydroactinidiolide. We observed positive responses from all cats, even several hours after
1060 applying 2 sprays of the tinctures. This finding suggested effective extraction of some of the cat-attracting

1061 single compounds in the tinctures. Indeed, when the amount of single compounds in the tinctures is
1062 expressed per gram dried plant material, these numbers surpass those obtained with the
1063 methanol:dichloromethane extraction method. However, we used 5 volumes of ethanol per 1 volume of
1064 plant tissue and therefore the quantity of single compounds per mL of tincture was relatively low (**Figure**
1065 **20**). Since roughly 1/5th of a milliliter of tincture was sprayed on the fabric, this implies that domestic cats
1066 are able to detect quantities of just a couple of micrograms.

1067 While the fruit galls of the silver vine plant already contained a large amount of actinidine, we found 3 ×
1068 more actinidine in the leaves, where it was also the dominant cat-attracting compound. Neonepetalactone
1069 and isoneonepetalactone were not detected in the leaves, but the other compounds were present in
1070 similar amounts when compared to the fruit galls. Interestingly, the response of cat Z where she would
1071 not touch the object was seen only for actinidine and the silver vine leaves. As expected, fewer
1072 compounds and lower quantities were detected in silver vine stem. Actinidine was still the most abundant
1073 compound, but four other compounds were found as well. A piece of silver vine stem harvested in East
1074 Asia (sold by Mew Neko, Austin, TX, USA) that was not used in the bioassays, but was found particularly
1075 popular among some of the cats (mostly cats N and Z) was analyzed as well and compared to the locally-
1076 grown, younger stem. The popular piece of silver vine stem contained neonepetalactone and more
1077 actinidine, but no dihydroactinidiolide was detected in the other stem.

1078 Discussion

1079

1080 In this study, we aimed to gain a better understanding of the "catnip response" in domestic cats by
1081 analyzing their behavior to different cat-attracting plants and chemically synthesized volatiles found in
1082 these plants. We observed differences between cats in their behavior to these plants and compounds that
1083 raise interesting questions about the way these compounds are perceived by cats and the underlying
1084 mechanism of olfactory sensation. We will address the most pertinent questions in more detail below.

1085 For this study, cats were exposed to cat-attracting plants and their volatile, active, single compounds for
1086 nearly 1,000 hours over a period of more than 2 years. To the best of our knowledge, this is the longest
1087 exposure of cats to catnip and catnip-like material ever documented. Following the final olfactory
1088 bioassay for this study in late 2020, *L. tatarica* wood and dried *A. polygama* leaves were made available
1089 to the cats in a nearly continuous manner. Since authors SB and EMB have been living together with the
1090 study cats before, during, and also after the study, we were able to closely monitor any potential negative
1091 health effects as the result of exposure to these olfactory stimuli. No adverse health effects, either
1092 physically or mentally, were observed, up to the publication date. These results support the current
1093 belief that these plants and their active compounds are safe (in the amounts that were available to them
1094 in this study) and offer an excellent source of environmental enrichment.

1095 Unfortunately, catnip is sometimes referred to as "kitty crack", the euphoric, blissful response to the cat-
1096 attracting plants considered a "high" or cats "tripping". This negative association can prevent cat
1097 guardians from offering olfactory enrichment. However, the plants that produce THC, the active
1098 compound in marijuana or weed (cannabis plant), cocaine or crack (coca plant), or heroin (opium poppy)
1099 are not related to any of the cat-attracting plants. Cannabis, the coca plant, and opium poppy are all
1100 species in families (Cannabaceae, Erythroxylaceae and Papaveraceae, respectively) that do not include
1101 the cat-attracting plant species (families: Euphorbiaceae, Actinidiaceae, Caprifoliaceae and Lamiaceae).
1102 Furthermore, the structures of THC, LSD, cocaine and heroin are much more complex than the cat-
1103 attracting iridoids and about twice the molecular weight (**Supplementary Figure 13A**). Two major
1104 differences between the above-described psychoactive drugs and the cat-attracting compounds are their
1105 route of entry and subsequent receptor binding. None of the psychoactive drugs are volatiles. The drugs

1106 need to end up in the blood (e.g., intravenous injection, orally, nasal tissue (snorting ≠ smelling), smoking)
1107 and subsequently bind receptors in the brain for them to be active; they do not elicit a response after
1108 smelling them. Nepetalactone only has an effect when the volatile is bound to the olfactory receptors after
1109 the cat inhaled air through the nose; it has no effect when absorbed into the blood after oral
1110 administration (Waller, Price and Mitchell 1969). Another difference between psychoactive drugs and cat-
1111 attractants is the duration of the response. While the “catnip response” lasts seconds to several minutes
1112 at most and can be easily interrupted, the effects of administering cocaine, heroin, LSD or smoking
1113 cannabis last for hours and cannot be stopped. Authors SB and EMB, who live with the cats, did not
1114 observe any withdrawal, abnormal behavior, or changes in behavior of any of the participating cats after
1115 the cat-attractants had been taken away from the cats. In the contrary, we believe we observed more
1116 positive interactions between cats in the testing area when the cat-attracting plants or single compounds
1117 were present, although this was not measured.

1118

1119 Much about the “catnip response” still seems riddled in mystery. We are clueless as to what the reason
1120 for, or biological function of, the response is and why it is only seen in felines. It has been hypothesized
1121 that a cat rubbing plant material with insect-deterrant compounds could reduce the number of mosquito
1122 bites (Uenoyama et al. 2021) and thereby prevent mortality due to mosquito-borne diseases. However,
1123 such diseases are uncommon in felines. Moreover, given the large range of mosquitoes in terms of both
1124 geographical spread and species that can serve as a host for blood meals, it would be likely that similar
1125 behavior would have evolved in other species. It is known that dihydroactinidiolide is present in secretions
1126 of the supracaudal and tail glands of the red fox, as well as in their urine. Our bioassays have
1127 demonstrated that domestic cats respond to dihydroactinidiolide. These observations justify revisiting the
1128 hypothesis that the “catnip response” is elicited by extreme quantities of compounds similar in structure to
1129 semiochemicals that serve in the communication between individuals from the same species
1130 (pheromones) (Todd 1963, Albone 1975).

1131 Another big unknown is which olfactory receptors are bound by the volatile cat-attracting molecules. Our
1132 study shows that some cats respond more strongly to actinidine than to lactones, while others do not
1133 respond to actinidine at all. This suggests that cats may have genetic differences in the receptor(s) that

1134 detect(s) these various compounds. Since the number of odorants far exceeds the number of olfactory
1135 receptors, it is believed that a single receptor can bind different odorants, but also that the same odorant
1136 can bind to different receptors, albeit probably with different affinities. This combinatorial olfactory
1137 receptor code and our poor understanding of structure-odor relationships make it difficult to speculate
1138 about the molecular mechanism that is involved in the “catnip response” and what might explain the
1139 difference in response between cats to the lactones and actinidine.

1140 The most obvious difference between actinidine and all other cat-attracting compounds is that actinidine
1141 contains a pyridine ring instead of a lactone, while still retaining the cyclopentane ring. These different
1142 features of actinidine may allow for binding to different receptors that are only expressed in some cats, or
1143 to mutated versions of the same receptors that bind the type I and II lactones.

1144 We found that the “catnip response” could not be elicited by catnip in cats habituated to silver vine or
1145 Tatarian honeysuckle. Since silver vine and honeysuckle both lacked *cis-trans*-nepetalactone, *trans-cis*-
1146 nepetalactone and dihydroactinidiolide, it may be possible that the other type I and II lactones in silver
1147 vine and Tatarian honeysuckle bind the same receptor(s) as nepetalactone and dihydroactinidiolide.

1148

1149 It has recently been shown that cats also respond to nepetalactol (Uenoyama *et al.* 2021), which is a
1150 reduced form of nepetalactone and similar in structure, but lacks the carbonyl group of the lactone
1151 (**Supplementary Figure 13B**). This suggests the carbonyl functional group may not be required for the
1152 lactones to engage with the receptor(s). Keesey and colleagues recently showed that *Actinidia arguta*
1153 leaves contain moderate amounts of nepetalactol, but only trace amounts of iridomyrmecin and
1154 actinidine, and no nepetalactone (Keesey *et al.* 2019). However, the *A. arguta* leaves that we tested
1155 previously did not elicit the “catnip response” in any of 8 domestic cats (Bol *et al.* 2017).

1156 Nelson and Wolinsky reported that cats responded positively to both iridomyrmecin and iso-iridomyrmecin
1157 (Nelson 1968), which is in agreement with the results from Uenoyama *et al.* and our findings. They also
1158 tested *cis-cis*-iridolactone (molecules XXV and XXVI in reference (Wolinsky *et al.* 1965)), epimers of
1159 iridomyrmecin and iso-iridomyrmecin that have not been tested by others (**Supplementary Figure 13B**).
1160 Surprisingly, none of the cats responded positively to the *cis-cis*-iridolactones, despite their structural
1161 similarity to iridomyrmecin and iso-iridomyrmecin (Nelson 1968). The only difference between these

1162 compounds is the methyl group on the cyclopentane ring, which is inverted on the *cis-cis* variants. This
1163 suggests that (the orientation of) this methyl group, along with the cyclopentane ring, might play an
1164 important role in binding to the receptors. While we did not see statistically significant differences in
1165 duration of the “catnip response” between compounds, the response to both neonepetalactone and
1166 isoneonepetalactone seemed lower than all other type I and II lactones. While the methyl group on the 5-
1167 membered ring is not inverted on neonepetalactone and isoneonepetalactone as it is on the presumably
1168 inactive *cis-cis*-iridomyrmecin and *cis-cis*-isoiridomyrmecin, it is planar to the cyclopentene ring. This
1169 planar orientation of the methyl group is different from all the other active type I and II lactones (**1 – 4, 7**
1170 and **8, Figure 12**) and may possibly account for the seemingly reduced response duration of the cats to
1171 neonepetalactone and isoneonepetalactone.

1172 Nelson and Wolinsky also found that cats responded positively to another compound that has not been
1173 tested by others: the bridged bicyclic matatabiether (**Supplementary Figure 13B**). Interpretation of the
1174 results from the work done by Nelson and Wolinsky is challenging however, because the experimental
1175 methods of the bioassays were not described in detail. Furthermore, no clear definition was given when a
1176 response was considered positive. Sniffing, licking or biting in the absence of head rubbing may have
1177 been considered a positive response (Katahira and Iwai 1975, Sakan et al. 1960).

1178

1179 We observed differences in attractiveness of plant materials to cats that may be explained by their
1180 chemical composition. The popularity of silver vine fruit galls and leaves may be explained by the
1181 presence of some compounds that were not detected in any of the other plants, the large number of cat-
1182 attracting compounds present, or the large quantity of actinidine. However, the combined results from the
1183 bioassays and GC-MS analysis also suggest there may be other, unidentified, cat-attracting compounds
1184 present in the cat-attracting plants, especially Tatarian honeysuckle. While no cat-attracting compounds
1185 were detected in any of the fragrances tested, the “catnip response” was seen twice after fabric with
1186 Drakkar Noir was made available to cat A. While cats O and V did not respond to actinidine, yet both cats
1187 responded to the Tatarian honeysuckle tincture, in which we only detected actinidine. We observed
1188 dishabituation of cat O to Tatarian honeysuckle, both after habituation to silver vine fruit galls (twice) and
1189 catnip leaves had occurred, while we were not able to detect any compounds in the honeysuckle that

1190 were not present or present at lower levels in the silver vine or catnip samples. The chemical composition
1191 of valerian root was similar to that of Tatarian honeysuckle and Indian nettle root. However, cat O
1192 responded significantly longer to Tatarian honeysuckle than to valerian root. Furthermore, cat Z did
1193 respond to Tatarian honeysuckle and Indian nettle root, but never to valerian root. Possibly, some plant
1194 samples contained odorants that had a repelling effect on some cats (e.g., valerian root). Indeed, the
1195 valerian root tincture did not contain any known cat-attracting compounds that were not also present at
1196 comparable levels in the dried valerian root, yet cat Z responded to the tincture and not to the dried plant
1197 material. Previous quantitation where compounds were extracted from plant tissues for not 2, but 7 days
1198 in dichloromethane yielded a similar pattern: none of the quantitated compounds was found to be only
1199 present or at higher levels in Tatarian honeysuckle compared to catnip and silver vine (Bol et al. 2017).
1200 Other cat-attracting single compounds may have been extracted, but not identified. Cats detect the
1201 volatile compounds emitted by the plant tissues in the air. For our chemical analysis we chose to extract
1202 the compounds from the plant tissues using solvents, to enable accurate identification and quantification,
1203 as was done in our previous work (Bol et al. 2017). There may be some discrepancy between naturally
1204 emitted volatiles from the plant materials and those that can be extracted with solvents. Headspace
1205 (airspace) analysis may provide results that better represent what cats detect than the solvent extraction
1206 methods, but most headspace analysis methods (SPME or purge and trap) cannot be easily quantified.
1207 Our quantification data however is a useful guide to the presence and relevant amounts of cat-attracting
1208 compounds present within each plant material tested in this study. A useful outcome of this study is that
1209 we were able to quantify a large group of cat-attracting compounds from the same plant materials that
1210 were exposed to the cats for behavioral analyses, enabling us to link some of the chemistry with cat
1211 behavior.

1212
1213 Our interest in cat-attracting plants ((Bol et al. 2017) and this work) originates from observations that cats
1214 A, N, O, V and Z did not respond to catnip prior to and during the tests done in 2016 (Bol et al. 2017),
1215 despite being exposed to the plant material longer and more frequently than any of the other cats in the
1216 study. Therefore, it was surprising that the same cats, especially all five, responded to catnip during the
1217 tests done for this work. In early 2016, cats A and Z were 9 and 5 years, respectively, and the three

1218 littermates were 16 months. It is often claimed that cats younger than 3 – 6 months do not respond to
1219 catnip. However, it might be possible that there is not a tight age cutoff, but that the “catnip response” is
1220 something that can or perhaps even needs to be acquired over time. Using the dataset from our 2017
1221 publication, we found that of young adult cats (6 – 18 months) almost 50% fewer responded to catnip
1222 than the older cats (**Supplementary Figure 14**). In contrast, the high percentage of cats responding to
1223 silver vine was equal among all 4 different age groups. Interestingly, the 5 cats in the study only started to
1224 respond to catnip months after they responded to silver vine. Long-term non-responders suddenly
1225 responding to catnip have been described in literature before, but for these cases it was suspected to be
1226 due to fluctuations in hormones of intact female cats (Todd 1963).

1227

1228 Our observations were done in a small group of cats. The advantage of this approach was that it allowed
1229 us to test relatively large numbers of cat-attracting plants and single compounds on the same group of
1230 domestic cats in a completely stress-free environment. The small, homogenous study population did not
1231 prevent us from obtaining answers to the main research questions. With this small group of cats, we were
1232 able to demonstrate that the individual cat’s response to other cat-attracting plants is similar to catnip, and
1233 that there is substantial variation between cats in the behavior during the “catnip response”. Furthermore,
1234 we learned that cats respond to a large number of type I and II lactones, but also to dihydroactinidiolide
1235 and actinidine. Based on results from this study and research recently done by Reiko Uenoyama and
1236 colleagues we now know that only 10 – 20% of domestic cats respond to actinidine. We were also able to
1237 demonstrate that the behavior to actinidine was different from the lactones. However, it is possible that
1238 we missed other differences that will only become apparent when larger numbers of domestic cats and a
1239 more heterogeneous population (e.g., different breeds) are tested. Another limitation of this study is that
1240 the position of the camera, both the angle and distance, made it sometimes difficult to observe certain
1241 activity in the testing area. For this reason, we were not able to study position of the ears and whiskers,
1242 pupil size and (excessive) salivation (Bol et al. 2017). Furthermore, the fixed camera position sometimes
1243 complicated discrimination between behaviors, e.g., licking or head rubbing. While it also would have
1244 prevented us from studying any delayed behavior in response to the olfactory stimuli that would have
1245 occurred off-camera, nothing indicated that such behavior did occur.

1246 Studying more cats, plants and single compounds will undoubtedly reveal additional cat-attracting plants
1247 and single compounds that can elicit the “catnip response”. Some of these molecules may affect even
1248 fewer cats than actinidine does. Newly identified cat-attracting volatiles do not necessarily need to come
1249 from plants. Both plagiolactone and gastrolactone (**Supplementary Figure 13B**) are similar in structure to
1250 the cat-attracting type I and II lactones, and are produced by different species of leaf beetles (Meinwald et
1251 al. 1977, Blum et al. 1978). We are not aware of any publication reporting the detection either
1252 plagiolactone or gastrolactone in plant tissue. It remains to be determined whether these two compounds
1253 can elicit the “catnip response”. Furthermore, it may be possible to synthesize many novel cat-attracting
1254 compounds that do not occur anywhere in nature.

1255
1256 In conclusion, we have performed a comprehensive study of the “catnip response” of domestic cats to five
1257 plants, 10 single compounds, and several other samples. We observed that while responses between
1258 cats were highly variable, the behavior of individual domestic cats to diverse cat-attracting plants as well
1259 as all lactones was quite similar. Interestingly, the response to actinidine was most divergent, with several
1260 non-responders and a small percentage of cats who preferred actinidine over all other compounds.
1261 Collectively, these results have increased our understanding of the “catnip response” in terms of both
1262 behavior and the chemical compounds that elicit it. It has also revealed potential differences in the
1263 perception of compounds between cats that warrant further investigation into the underlying genetics of
1264 cat odorant perception and the mechanism(s) of action of these compounds.

1265 Acknowledgements

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1270 Biodiversity, Conservation and Attractions) for providing lyophilized *Acalypha indica* roots. We also thank
1271 Frances Mae Tenorio and Maria Brink for help with the analysis of the cat behavior.

1272

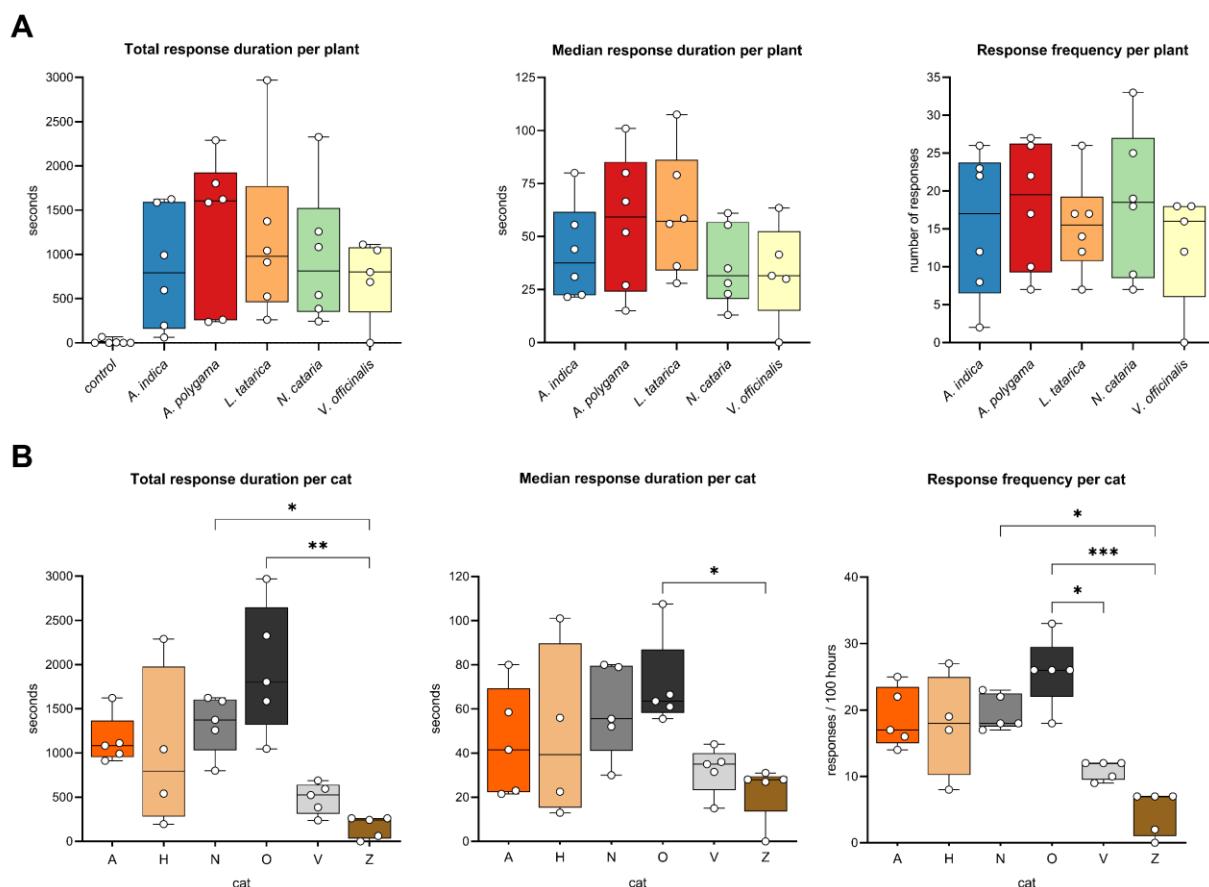
1273

1274 Author contributions

1275 **SB** conceived, designed, and coordinated the study, performed experiments, collected and analyzed data
1276 in GraphPad Prism and BORIS, and wrote the manuscript. **AS** synthesized the single compounds,
1277 extracted and performed chemical analysis of the samples, created Figure 12, Supplementary Figure 13,
1278 and edited the manuscript. **EMB** assisted with experiments, created Supplementary Figures 3 and 7, and
1279 edited the manuscript. **GRF** extracted and performed chemical analysis of the samples, analyzed the
1280 data, and edited the manuscript.

1281 **Figures**

1282

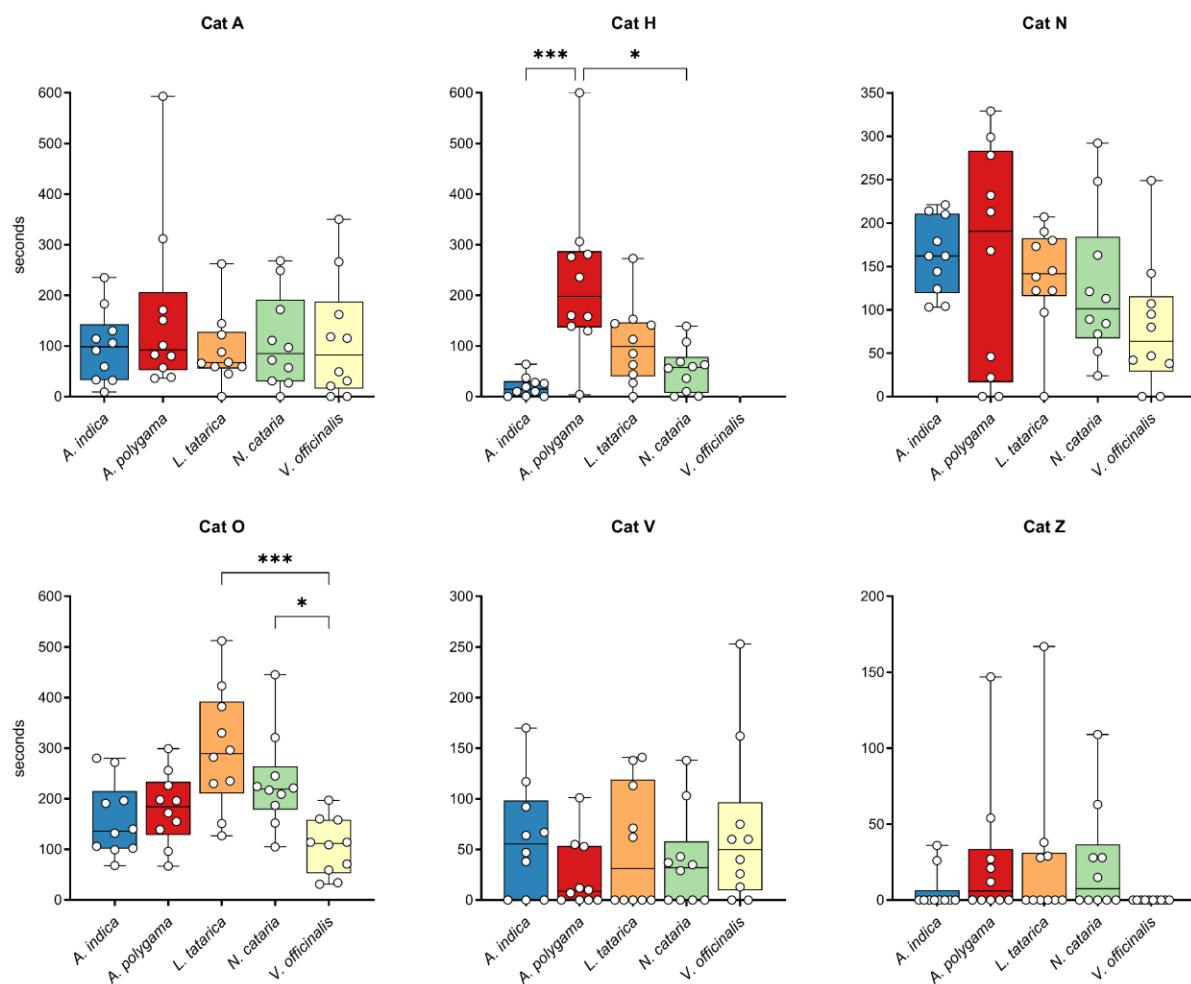


1283

1284 **Figure 1. Response duration and response frequency of domestic cats to cat-attracting plants.**

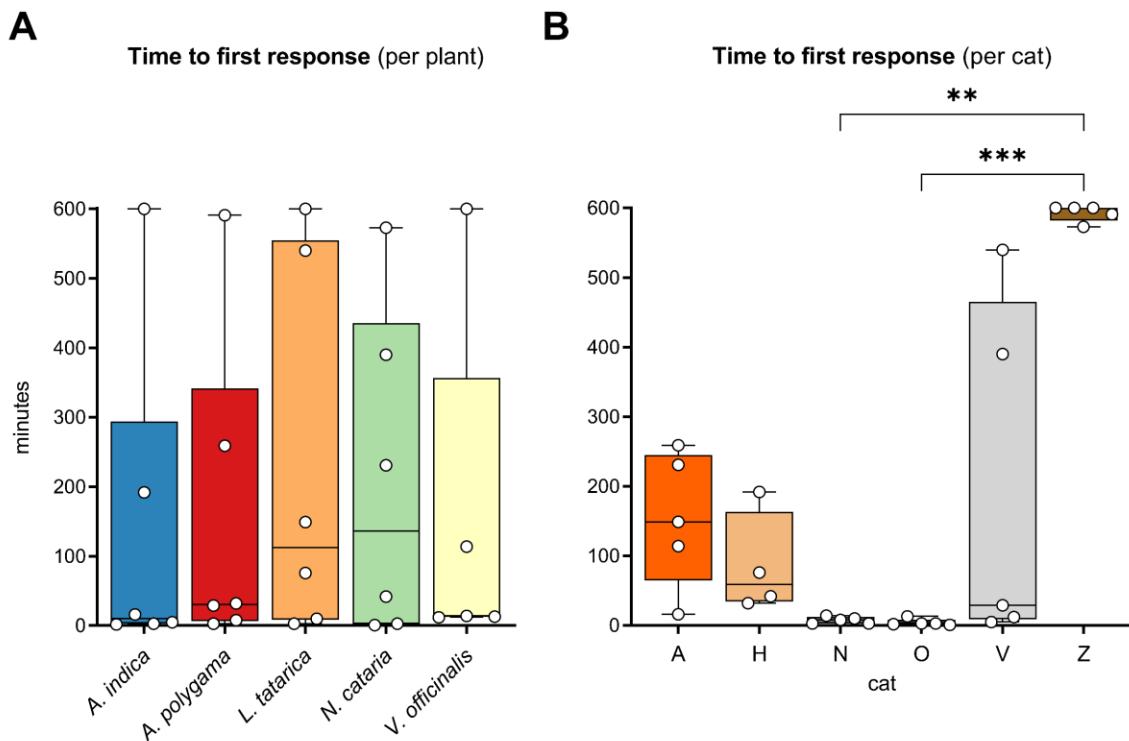
1285 Box and whisker plots showing the total response time, median response duration, and the total number
1286 of responses of 6 domestic cats to 5 cat-attracting plants. Each dot represents the data of one cat; the
1287 middle line in the bars shows the median value. Each cat-attracting plant was available for 100 hours, the
1288 control (green tea) was available for 500 hours (100 hours for each of the 5 plants tested). (A) Data
1289 shown per plant. Note the large spread of the data points, indicating large variation in response duration
1290 and frequency to the various plants between the cats. Differences between the 5 plants (total response
1291 duration, median response duration and response frequency) were not statistically significant ($P > 0.05$,
1292 mixed-effects repeated measures ANOVA and Tukey post-hoc test, corrected for multiple comparisons).
1293 We obtained 5 instead of 6 data points for *V. officinalis* since cat H was unable to participate due to

1294 medical reasons. For the statistical analysis of the paired data with missing data (cat H) we used a
1295 parametric test (mixed-effects repeated measures ANOVA). Therefore, for the analysis we used the
1296 average values (both the average response time to a plant for each cat and the average of the cats for
1297 each plant) instead of the median. Using either the average or median data did not affect the outcome of
1298 the statistical analysis. **(B)** Differences in total response time, median response duration, and response
1299 frequency between cats. Colors represent the fur color of the cats. Response duration and frequency
1300 differed significantly between the cats (Kruskal-Wallis). P values shown in the graph are from Dunn's
1301 post-hoc tests. * P < 0.05; ** P < 0.01, *** P < 0.001



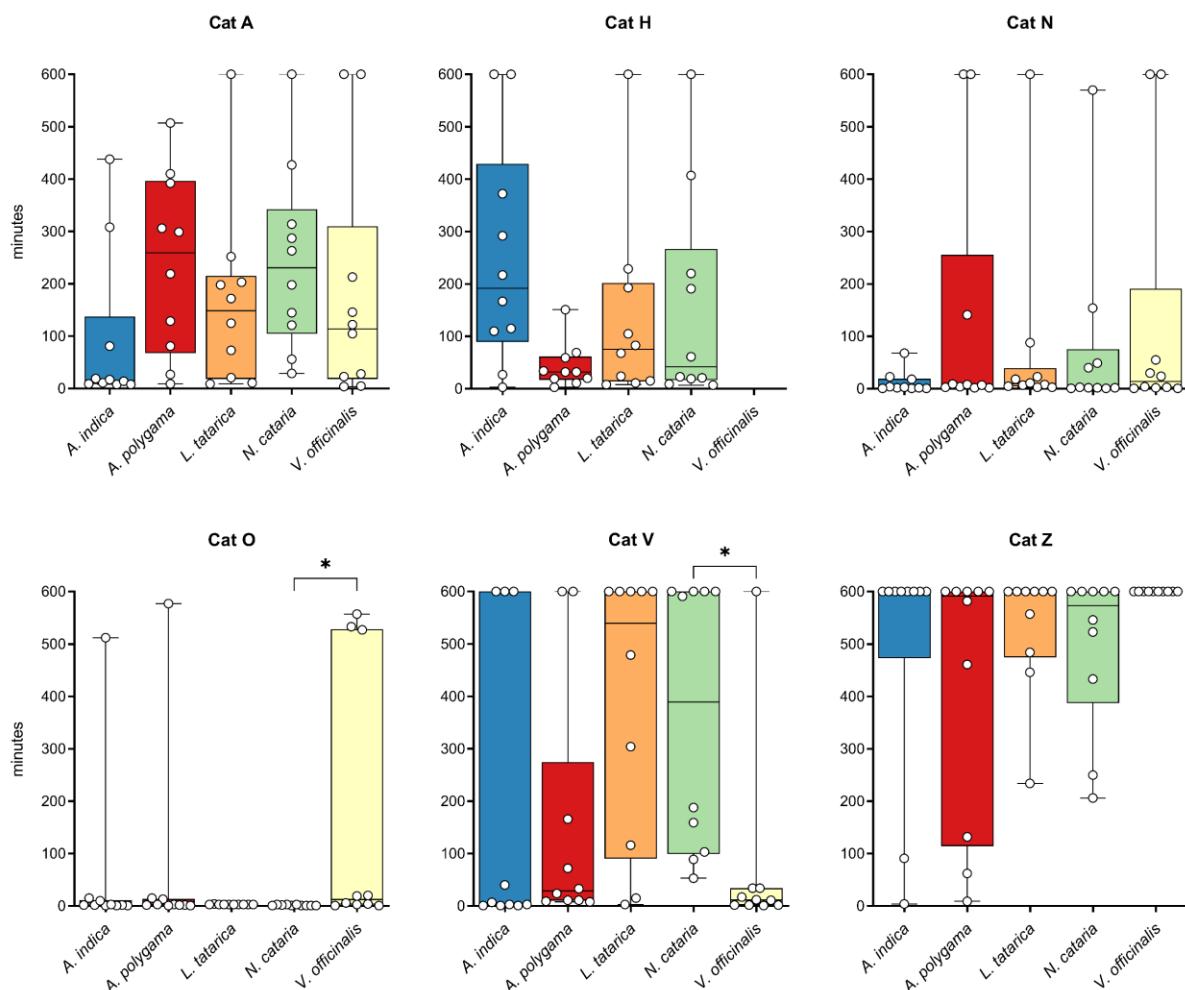
1302

1303 **Figure 2. Response duration to cat-attracting plants shown for each cat individually.** Each dot
1304 represents the total response duration of one day (10 hours), with the middle line in each box showing the
1305 median of these 10 days. Each plant was available for 10 days (total of 100 hours). Note that the Y axes
1306 are not the same for all graphs since the goal was to illustrate differences between the plants for each
1307 cat, not between cats. The Kruskal-Wallis test was used to test for statistically significant differences
1308 between plants. P values shown in the graph are from Dunn's post-hoc tests. * P < 0.05; *** P < 0.001



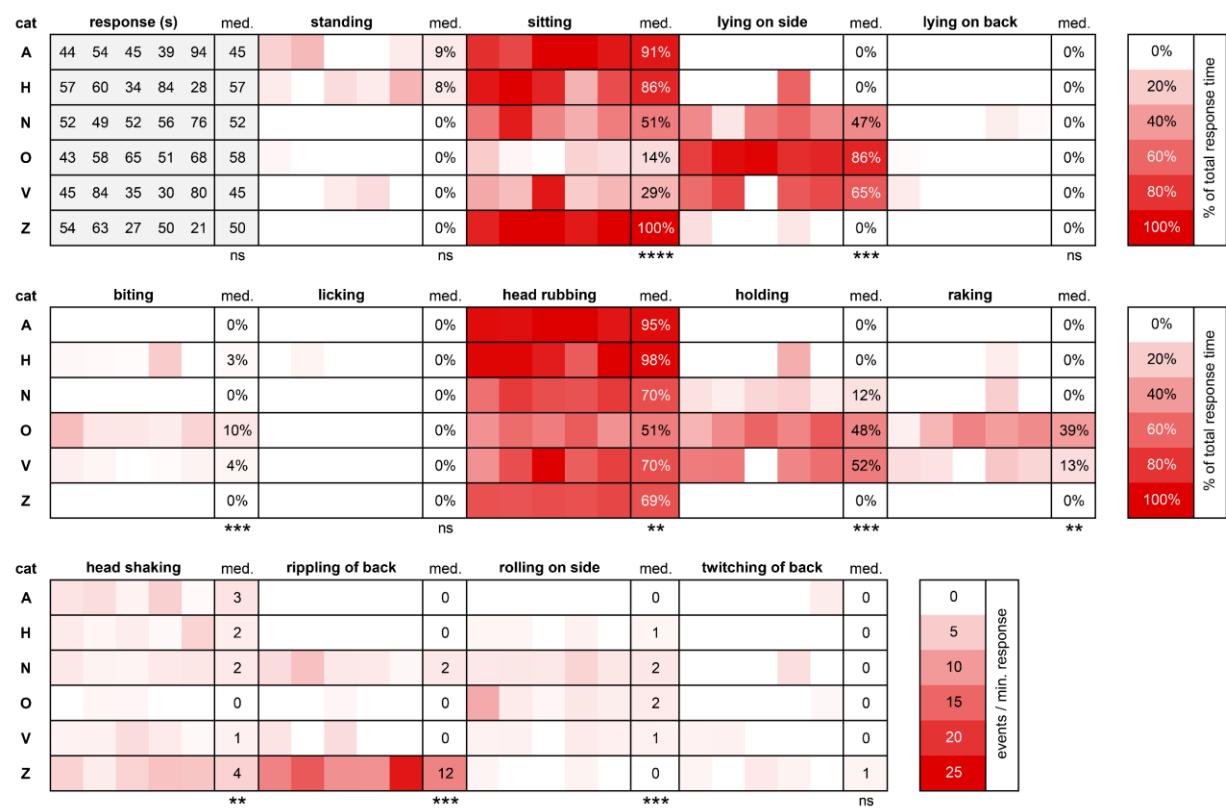
1309

1310 **Figure 3. Time to first response.** (A) The median time till the first response of 6 cats is shown for 5 cat-
1311 attracting plants. Each dot represents the median time till the first response of 10 testing days of each cat
1312 to the cat-attracting plants. Cat H did not participate in testing *V. officinalis*. There were no statistically
1313 significant differences in the time to the first response between the plants ($P > 0.05$, mixed-effects
1314 repeated measures ANOVA (paired test with missing data; see **Figure 1A**)). (B) The median time till the
1315 first response of 5 cat-attracting plants is shown for the 6 domestic cats. Each dot represents the median
1316 time to first response of 5 cat-attracting plants. The differences between the cats were statistically
1317 significantly different (Kruskal-Wallis). P values shown in the figure are from Dunn's post-hoc test. ** $P <$
1318 0.01 , *** $P < 0.001$



1319

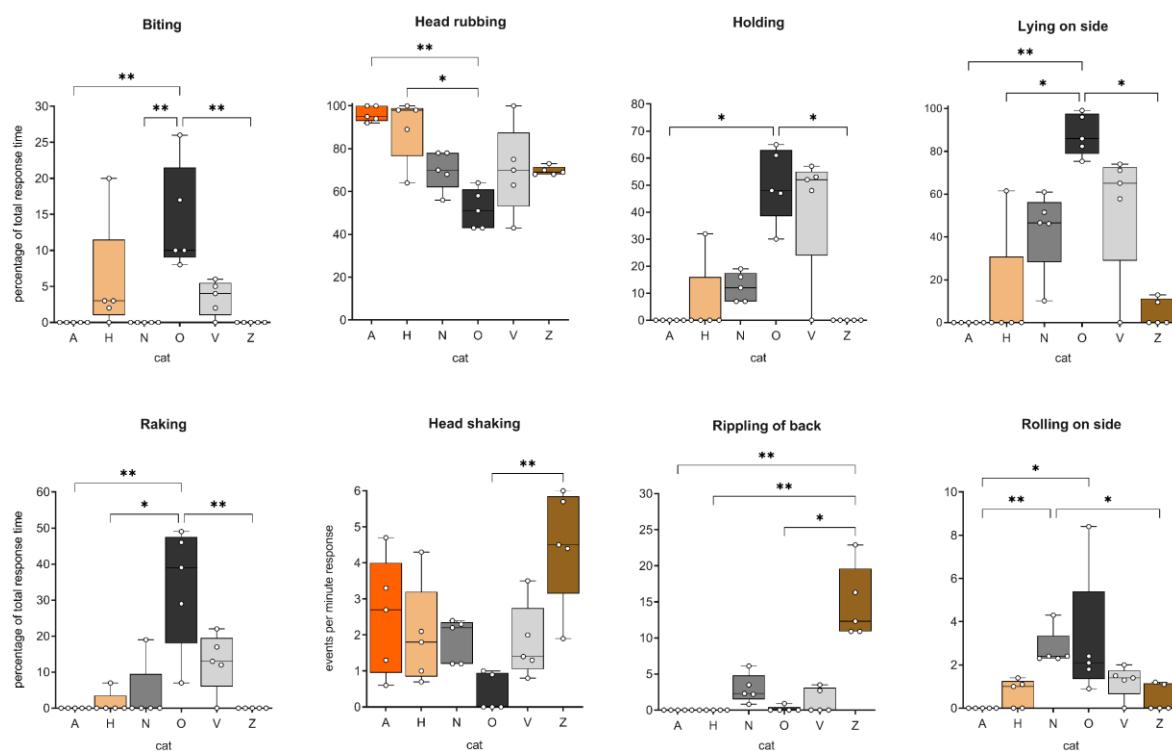
1320 **Figure 4. Time to first response to 5 cat-attracting plants shown for each cat separately.** Each dot
1321 shows the time it took the cats for their first response on each of the 10 test days. Cat H did not
1322 participate in the testing of *V. officinalis*. * $P < 0.05$



1323

1324 **Figure 5. Heatmap showing similarities and differences in behavior between 6 domestic cats in**
 1325 **response to *N. cataria* (catnip).** For each cat, the five responses nearest to 60 seconds were analyzed
 1326 using BORIS behavioral analysis software. All P values shown are from the Kruskal-Wallis test. med,
 1327 median; ns, not statistically significantly different; ** P < 0.01; *** P < 0.001; **** P < 0.0001

1328

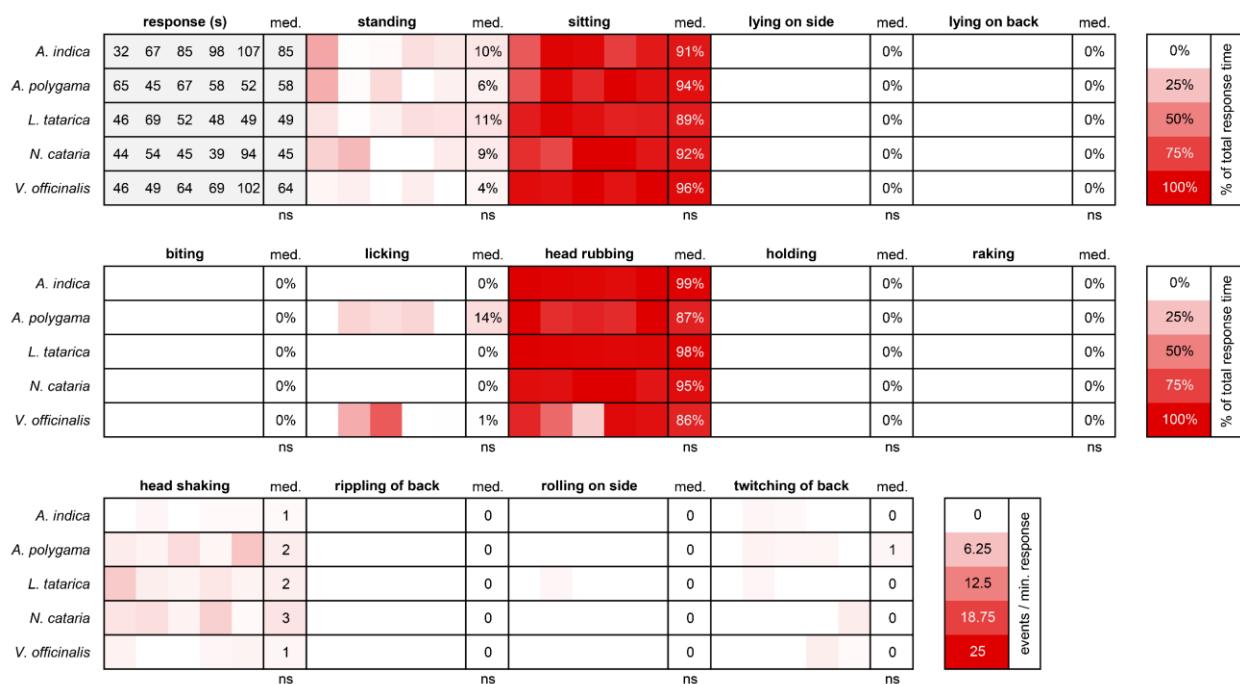


1329

1330 **Figure 6. Body position and behavior of 6 domestic cats observed in response to *N. cataria***

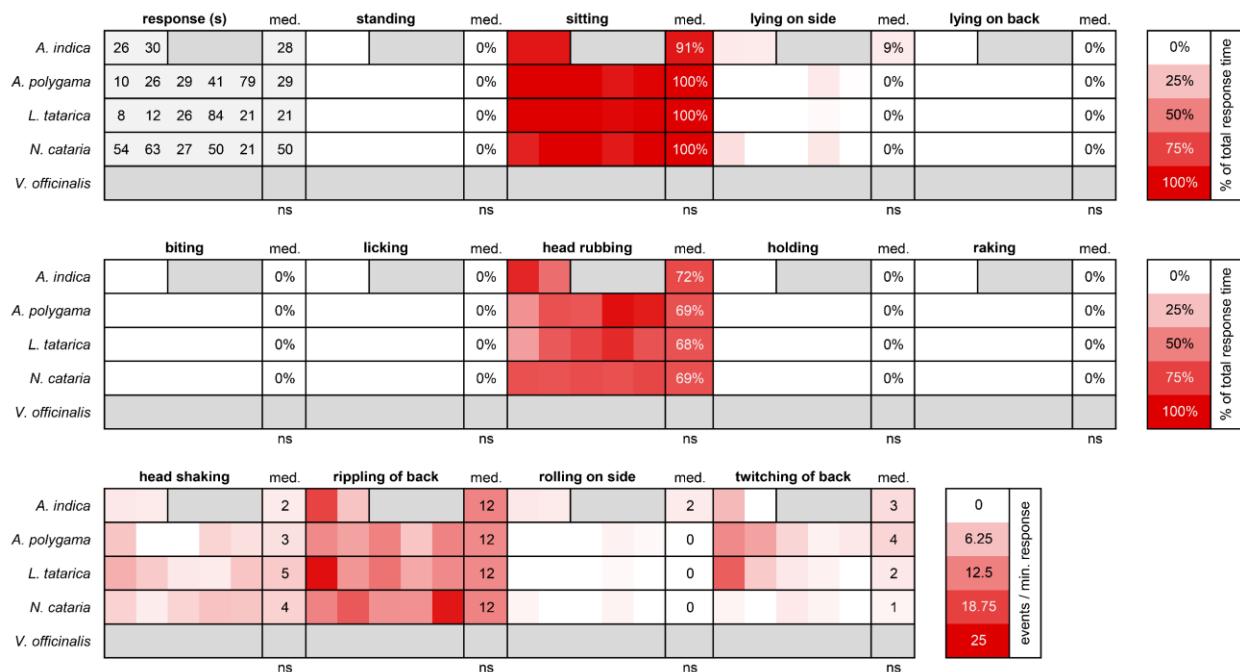
1331 **(catnip).** Results for “biting”, “head rubbing”, “holding”, “lying on side”, and “raking” are shown as time
1332 spent relative to the total response duration (percentage), whereas results for “head shaking”, “rippling of
1333 back” and “rolling on side” are depicted as the number of events per minute of response. Data for the
1334 body position “sitting” is not shown because sitting and lying down were mutually inclusive and inversely
1335 correlated (Figure 5). All P values shown are from Dunn’s post-hoc tests. * P < 0.05; ** P < 0.01

Cat A

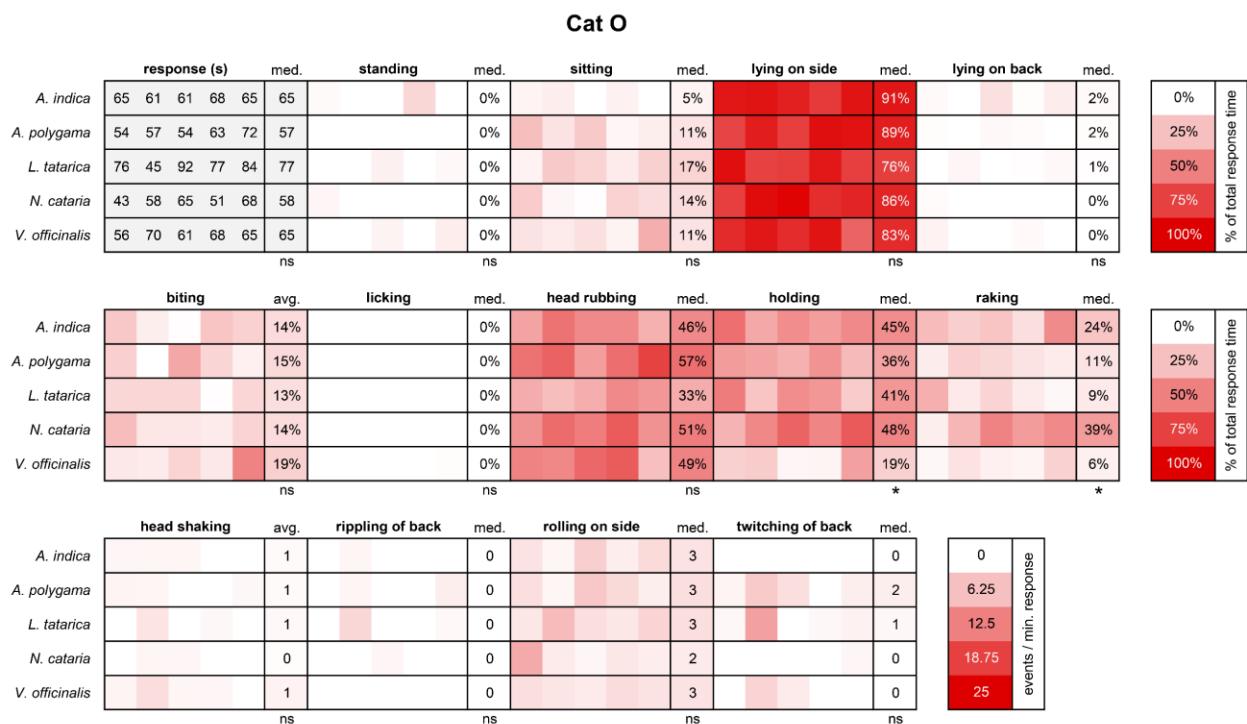


1336

Cat Z

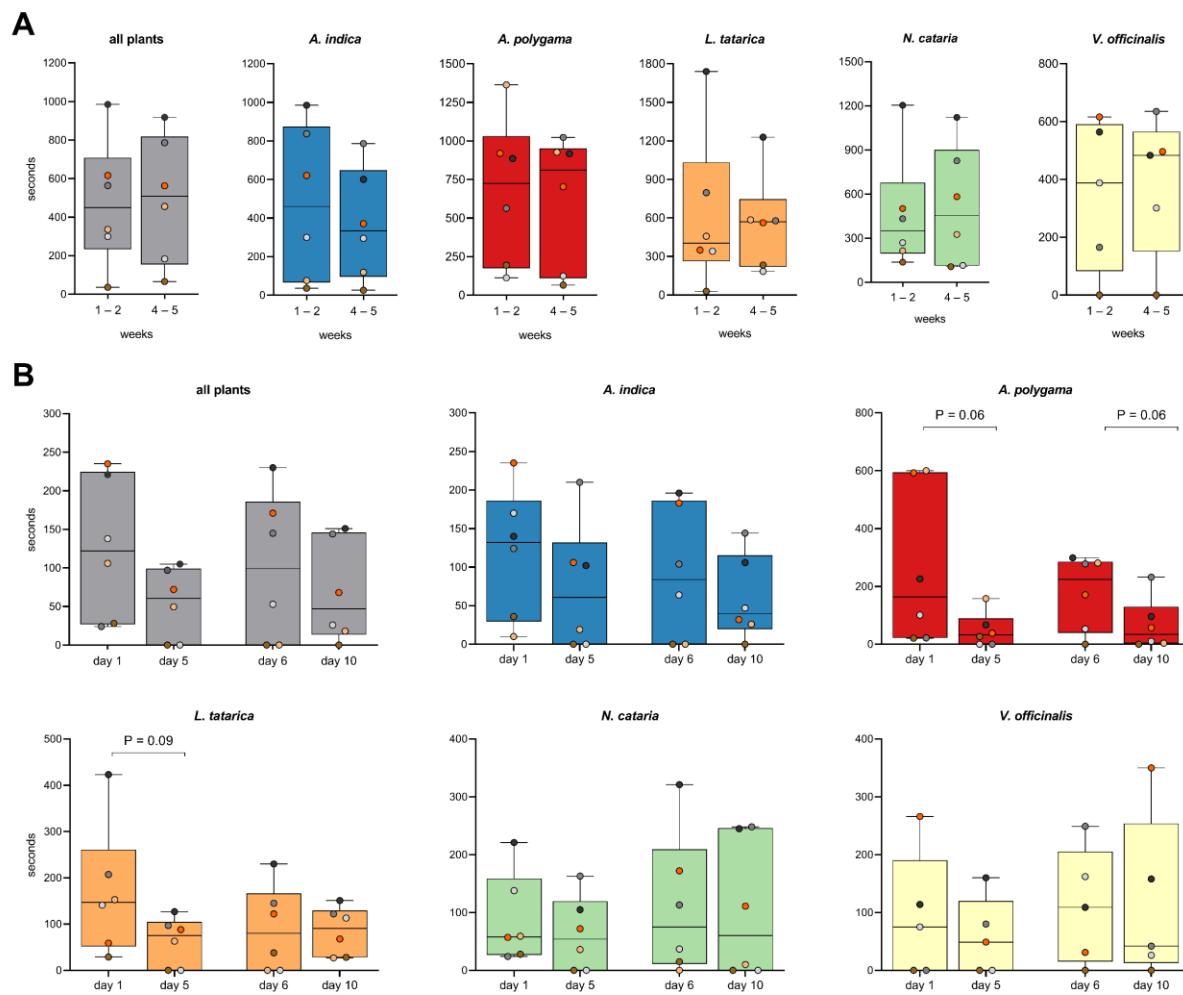


1337



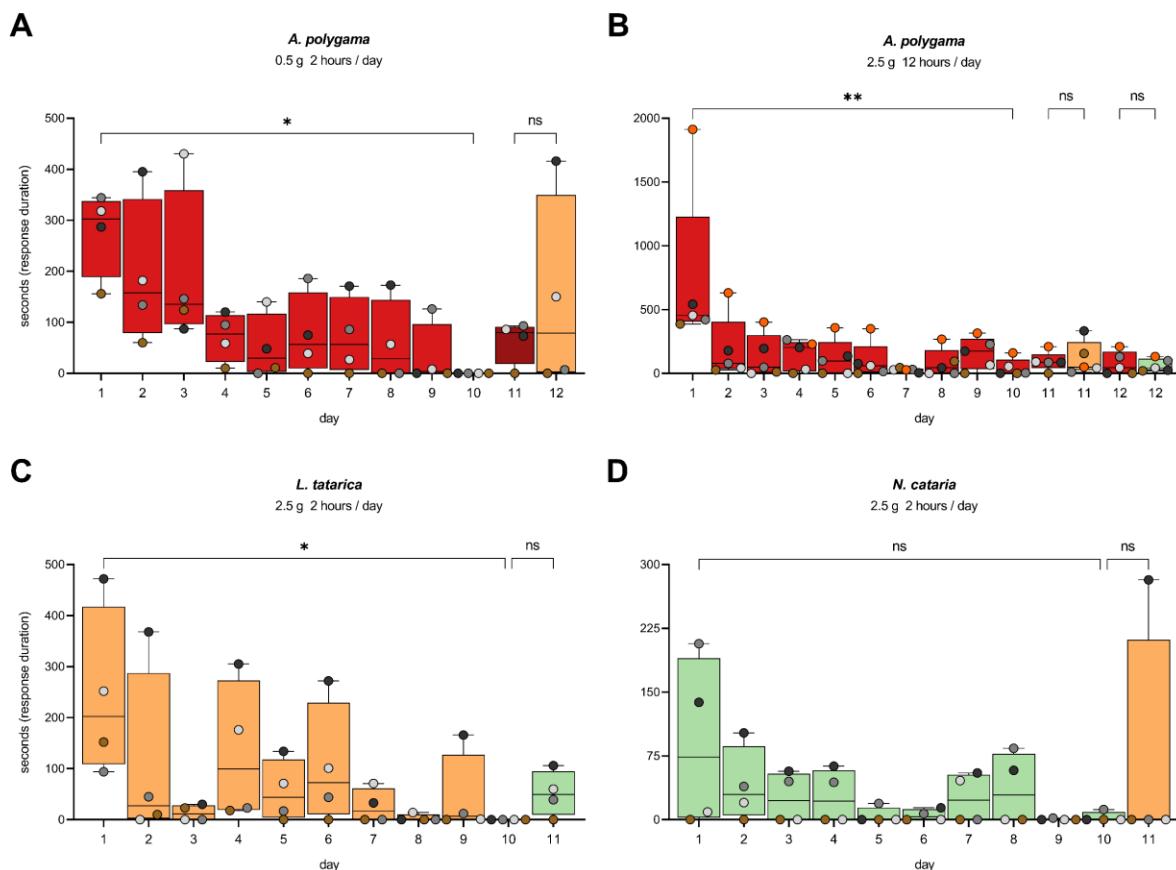
1338

1339 **Figure 7. Body position and behavior observed during the response to various cat-attracting**
1340 **plants.** For cats A, Z and O (Figure 7**A**, **B** and **C**, respectively), the five responses nearest to 60 seconds
1341 were analyzed using BORIS behavioral analysis software. We observed only two responses from cat Z to
1342 *A. indica*. Therefore, two responses instead of 5 were analyzed. P values shown are from the Kruskal-
1343 Wallis test. med, median; ns, not statistically significantly different; * P < 0.05



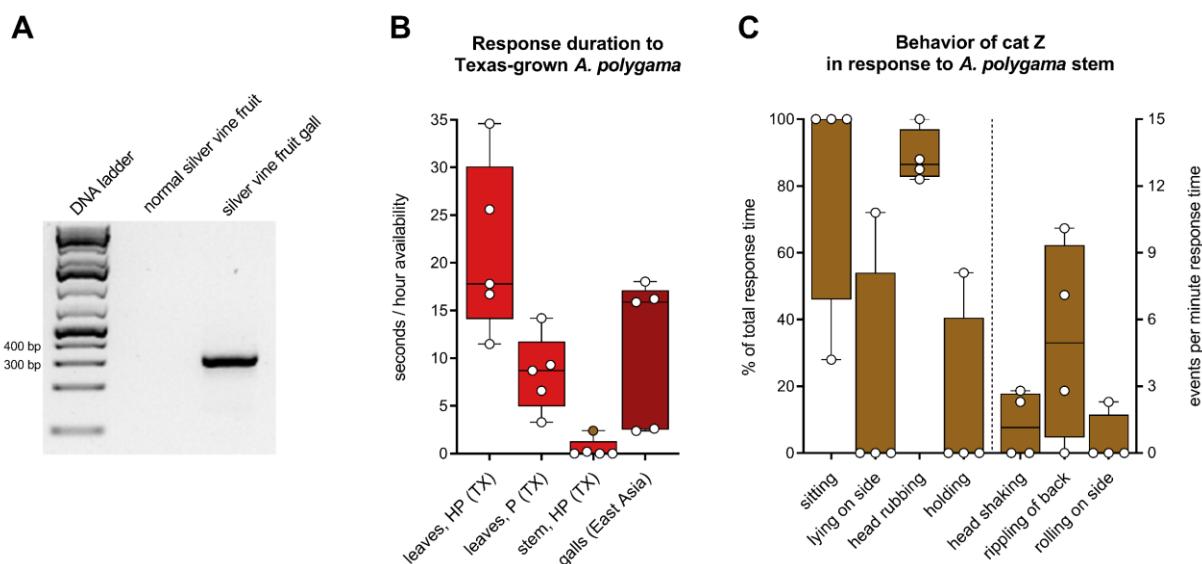
1344

1345 **Figure 8. Response duration to cat-attracting plants over time.** Each dot represents data (total
 1346 response time) of one cat. When all plants were compared, each dot shows the median value of the total
 1347 response durations to the 5 cat-attracting plants. **(A)** The total response duration of 6 cats to 5 cat-
 1348 attracting plants during the first 5 testing days (50 hours; weeks 1 – 2) was compared to the total
 1349 response time during the 5 testing days (50 hours) during weeks 4 – 5 (**Supplementary Figure 2A**).
 1350 The test periods of two weeks were separated by a 9-day interstimulus interval. **(B)** Total daily response
 1351 time of 6 cats during the first (day 1 and 6) and last day (days 5 and 10) of both two-week testing periods.
 1352 Cat H did not participate in testing *V. officinalis*. For all statistical analyses the paired, non-parametric
 1353 Wilcoxon matched-pairs signed rank test was used. All P values were > 0.05. Only P values < 0.1 are
 1354 shown.



1355

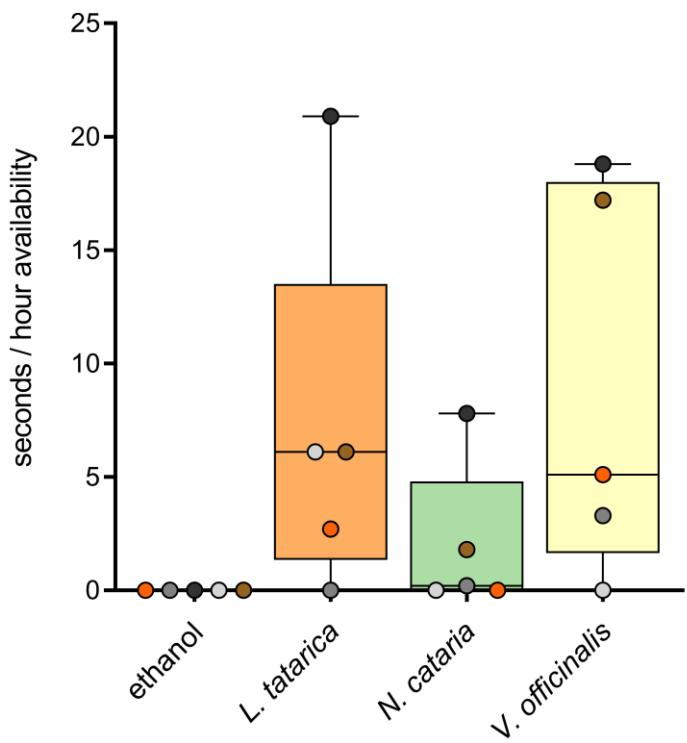
1356 **Figure 9. Habituation and dishabituation to cat-attracting plants.** The response duration of 4 – 5
1357 domestic cats to three different cat-attracting plants is shown for 10 consecutive days. With habituation a
1358 gradual decrease in response duration over time is seen. Dishabituation is the reappearance or increased
1359 duration of a response to a different stimulus that is offered to the cats after habituation has occurred (day
1360 11 or 12, or both) and its duration is similar or higher to what was seen on day one. Results for *A.*
1361 *polygama* (**A** and **B**) are shown in red, for *L. tatarica* (**C**) in orange, and for *N. cataria* (**D**) in green. See
1362 **Supplementary Figure 2B** for more details. The differences between day 1 and 10 for *A. polygama* and
1363 *L. tatarica* were statistically significant (Friedman test). P values shown in the figure are from Dunn's post-
1364 hoc test. * P < 0.05; ** P < 0.01



1365

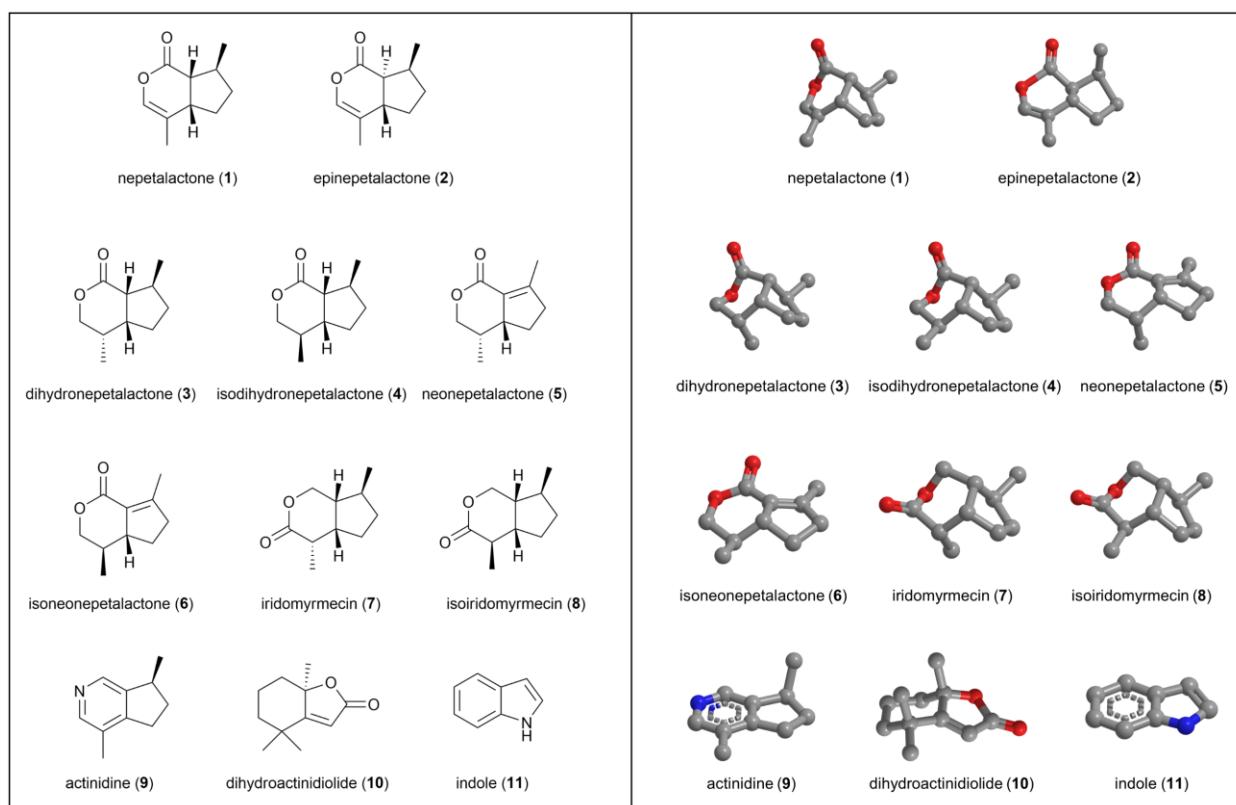
1366 **Figure 10. Response of domestic cats to Texas-grown *A. polygama*.** (A) Detection of *P. matatabi*
1367 DNA in dried *A. polygama* fruit galls from East Asia. Species-specific primers were used to amplify a 330
1368 bp fragment of the mitochondrial cytochrome oxidase subunit 1 gene. Sanger sequencing and nucleotide
1369 BLAST confirmed the DNA was from the gall midge *P. matatabi*. (B) Response time, shown in seconds
1370 per hour availability, of 5 cats to Texas-grown silver vine plant material. The cats were offered dried
1371 leaves from a female and male silver vine variety (Hot Pepper and Pavel, respectively), as well as dried,
1372 lignified stem. The response time to dried, powdered *A. polygama* fruit galls originating from East Asia is
1373 shown in dark red. Hot Pepper and Pavel leaves were available to the cats for 15 and 16 hours,
1374 respectively. Stem was available 2 × 15 hours. Powdered silver vine galls were available for 100 hours
1375 total (10 days, 10 hours per day). (C) Observed behavior of cat Z in response to Texas-grown *A.*
1376 *polygama* stem (brown dot in panel B). Bars show either behavior expressed as the percentage of the
1377 total response time (left Y axis) or the number of events per minute response time (right Y axis; “head
1378 shaking”, “rippling of back”, and “rolling on side”). Cat Z responded 4 times to the locally grown silver vine
1379 stem, with a total response time of 74 seconds. Only observed behavior is shown. HP, *A. polygama* Hot
1380 Pepper variety; P, *A. polygama* Pavel variety; TX, Texas

Response duration to tinctures



1381

1382 **Figure 11. Response time of domestic cats to tinctures made from cat-attracting plants.** Box and
1383 whisker plot showing the median response time of 5 cats (horizontal line) and median response time of
1384 each cat (dots). The response time is shown as time per hours availability of the tinctures. Each tincture
1385 was available for 5 hours. Ethanol was used as a negative control. The response duration of cat Z to the
1386 V. officinalis tincture is shown as a brown dot (18 seconds/hour availability). This cat did not respond at all
1387 to 15 g dried valerian root that was available for 10 days, 10 hours per day.

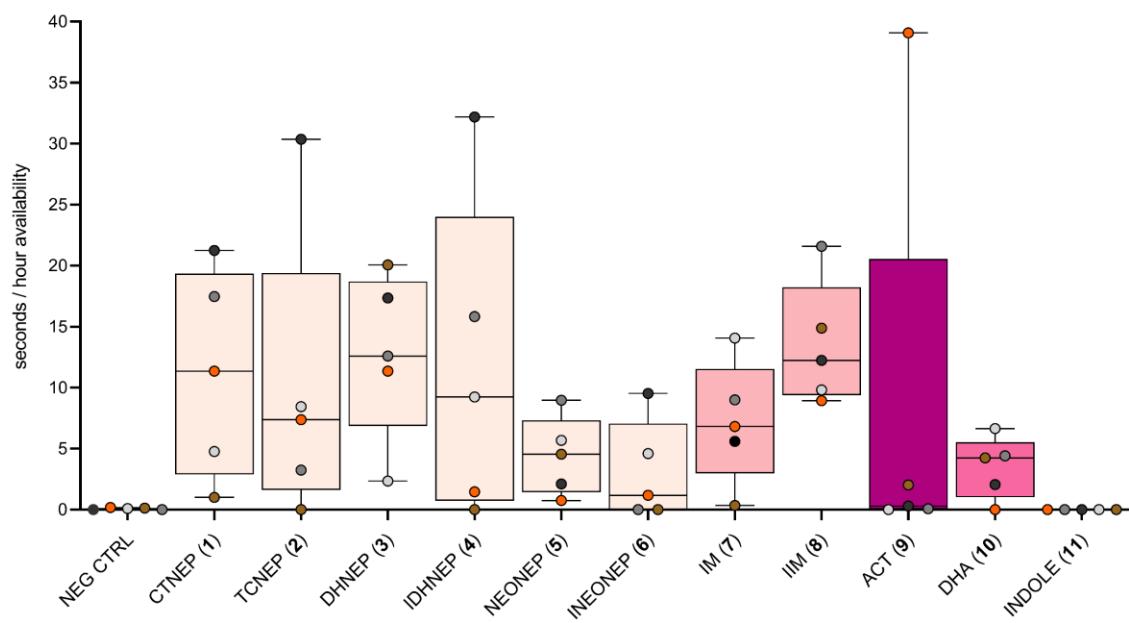


1388

1389 **Figure 12. Structures of the single compounds used for bioassays with domestic cats.** Two
1390 dimensional structures are shown on the left, 3D structures are shown on the right. Oxygen atoms are
1391 shown in red, nitrogen in blue. Nepetalactone (1) and epinepetalactone (2) are also referred to as *cis*-
1392 *trans*-nepetalactone and *trans-cis*-nepetalactone, respectively. Note how the location of the carbonyl
1393 group is different between the type I lactones 7 – 8 and the type II lactones 1 – 6.

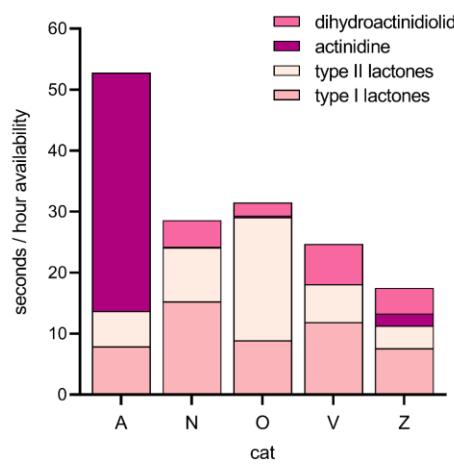
A

Total response duration to single compounds



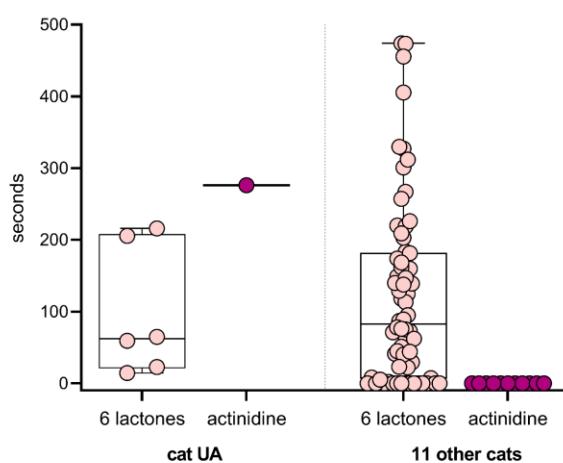
B

Total response duration to single compounds



C

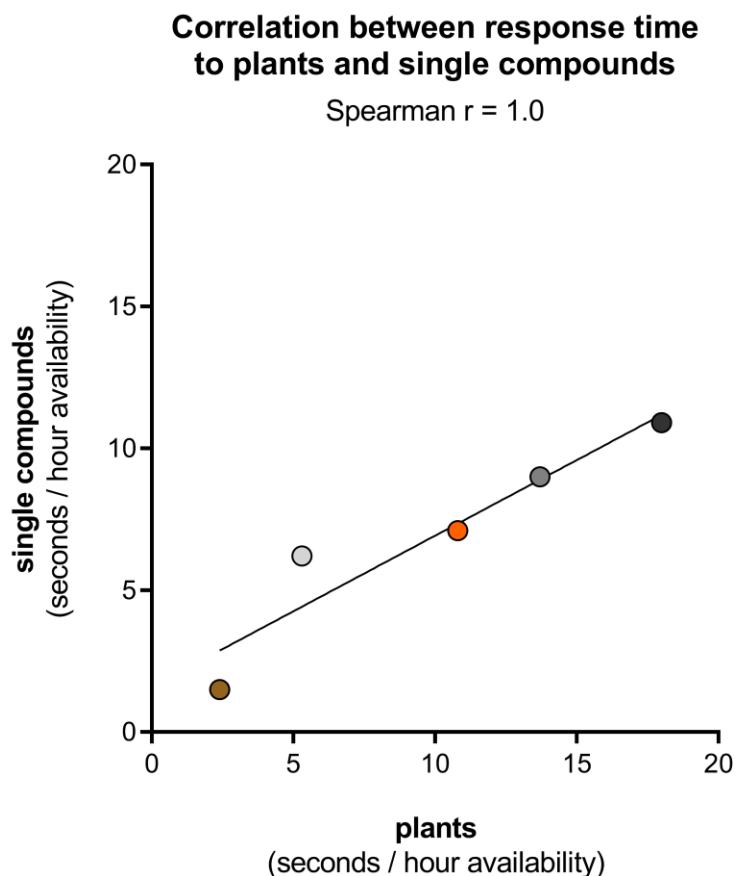
Head rubbing & rolling



1394

1395 **Figure 13. Response time of domestic cats to single compounds. (A)** Response time, shown as
 1396 seconds per hour each compound was available, per compound. Note the large range and uneven
 1397 distribution of the data for actinidine. Each compound was available for at least two days; 5 hours on the
 1398 first day and 12 hours on the subsequent test day. Negative controls (fabric with evaporated diethyl ether)
 1399 were always tested alongside the single compounds. **(B)** Response time to single compounds, grouped
 1400 by their chemical structure, shown per cat. For a cat responding equally long to every class of compounds

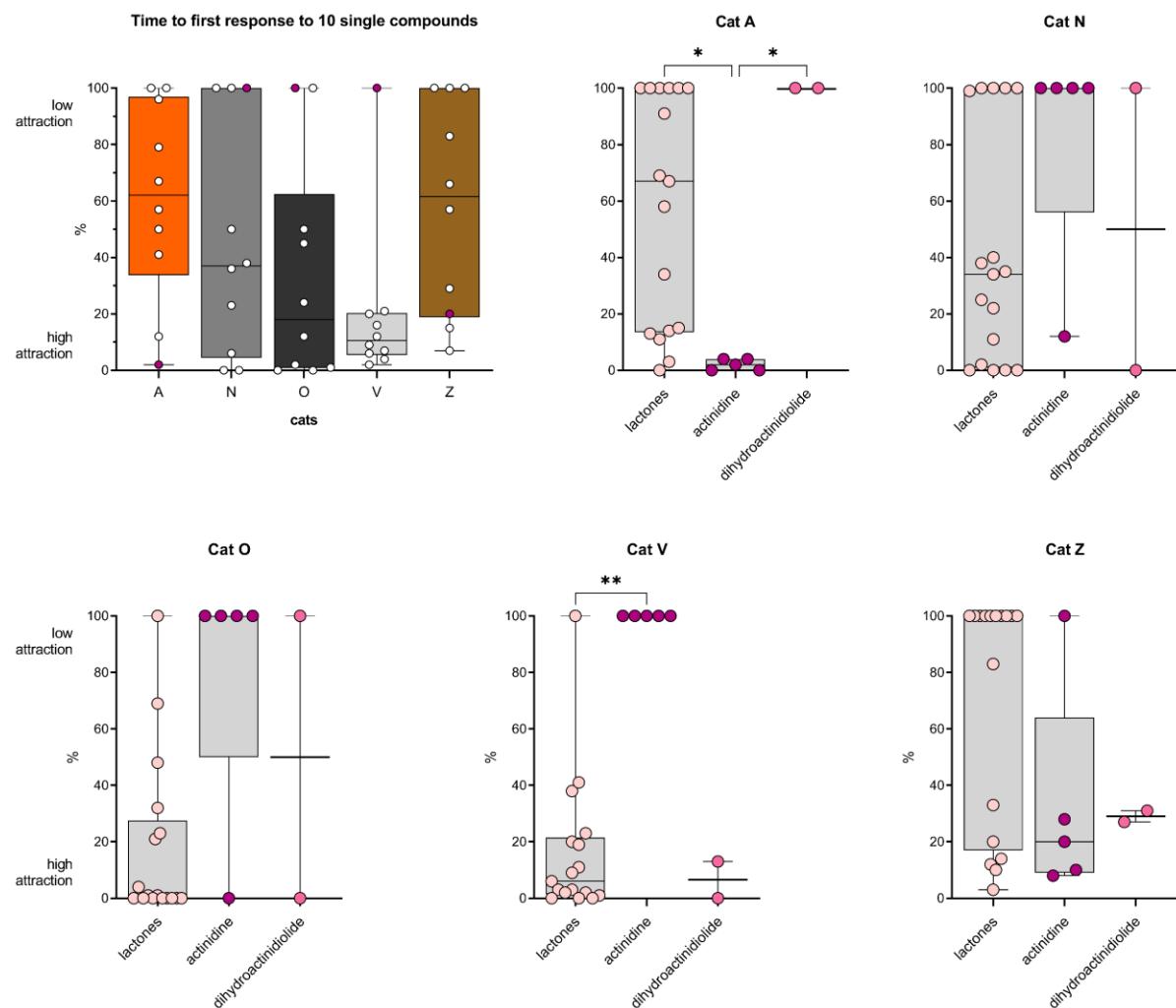
1401 (assuming there is no variation in response to the compounds within each group), one would see equal
1402 heights for each of the 4 portions of the bar. Type I and II lactones were available for 34 and 120.5 hours,
1403 respectively. Actinidine was tested for 53 hours on 5 days and dihydroactinidiolide was available for the
1404 cats for a total of 17 hours (2 days). **(C)** Duration of head rubbing and rolling of 12 domestic cats in
1405 response to iridoids. The data plotted here was obtained from the supplementary online material recently
1406 published by Uenoyama *et al.* (Uenoyama et al. 2021). The authors did not analyze or discuss these data
1407 in their article. The name of the only cat responding to actinidine in the study of Uenoyama *et al.*
1408 coincidentally is also cat A and is not the same cat as cat A in our study. To avoid confusion, we renamed
1409 this cat UA.



1410

1411 **Figure 14. Correlation between response duration to cat-attracting plants and single compounds.**

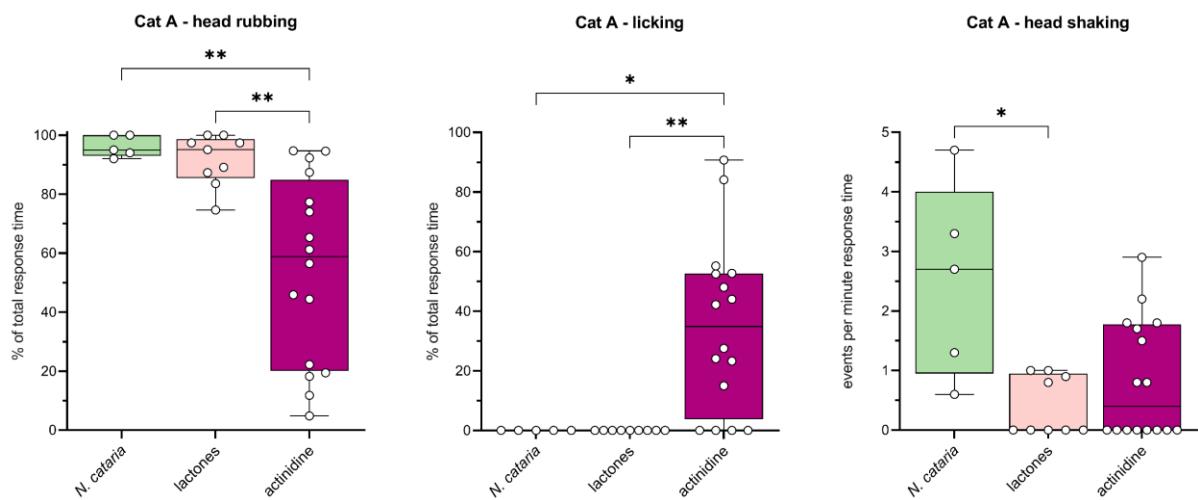
1412 For each cat the median of the 5 response times to the 5 cat-attracting plants (X axis) and the median of
1413 the 10 response times to 10 single compounds (1 – 10) (Y axis) are shown.



1414

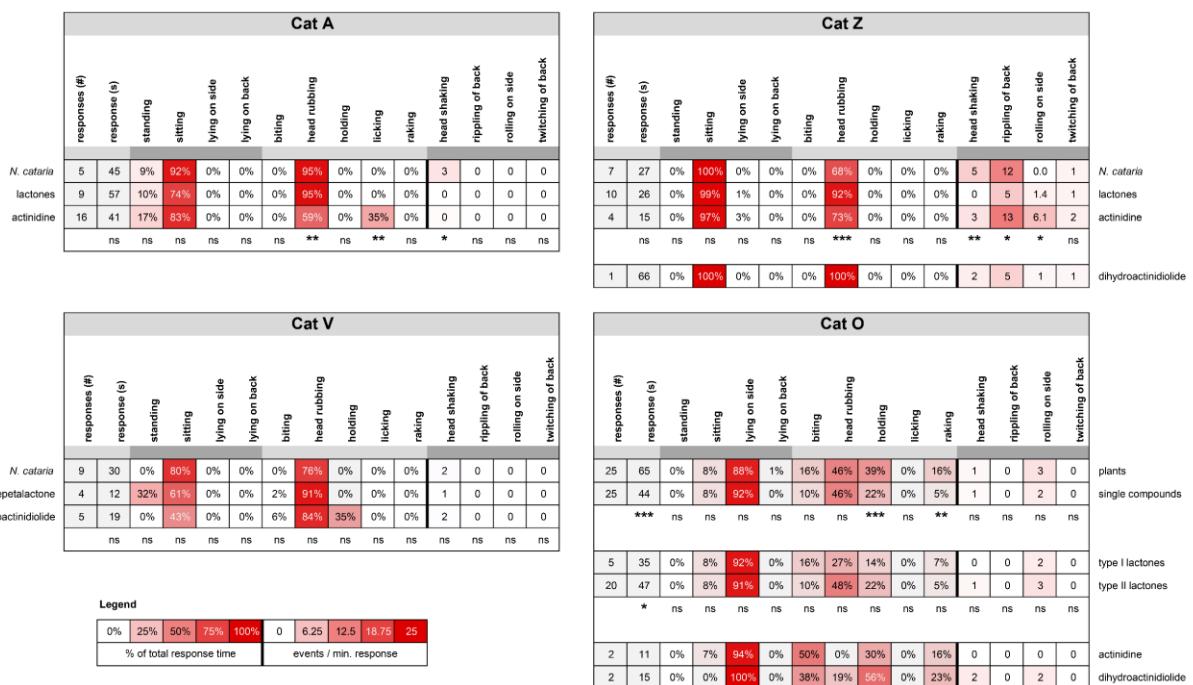
1415 **Figure 15. Time to first response of 5 domestic cats to single cat-attracting compounds.** The time
1416 to first response was determined for every cat, for every day that a single compound (1 – 10) was tested
1417 (n=24). When a cat did not respond to a compound on a test day, the time the stimulus was available that
1418 day was used as time to first response. Since the compounds were available for different durations,
1419 typically 5 and 12 hours, the time to first response was expressed as a percentage of the time the
1420 compound was available, with 0% being an immediate response and 100% no response at all that day.
1421 For each compound (10 per cat) the median percentage is shown. The second test day of
1422 neonepetalactone was not included because the recording stopped about 40 minutes after the start of the
1423 experiment. The differences in time to first response between the 5 cats was statistically significant ($P <$
1424 0.05, Friedman test). In addition, the differences in time to first response between actinidine and other

1425 compounds for cat A, as well as the difference between the lactones and actinidine for cat V were
1426 statistically significant (Kruskal-Wallis test). P values shown in the figure are from Dunn's post-hoc test. *
1427 P < 0.05; ** P < 0.01



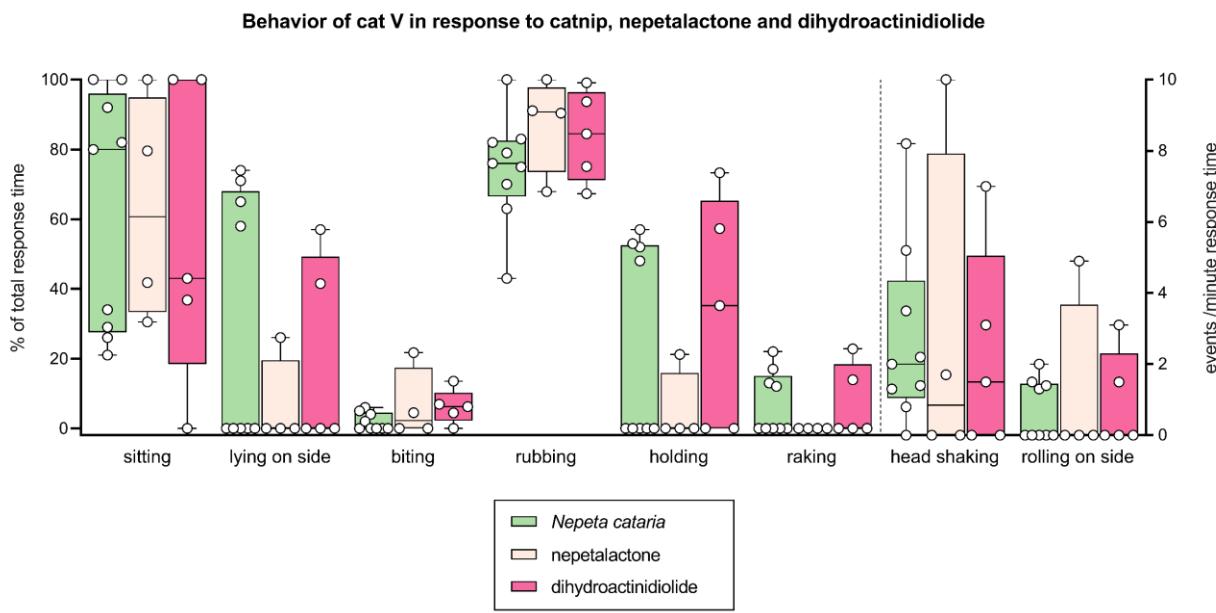
1428

1429 **Figure 16. Differences in behavior of cat A between responses to actinidine, lactones and *N.***
1430 ***cataria*.** Nine responses to the lactones and 16 responses to actinidine with a response duration 30 – 90
1431 seconds were analyzed using BORIS behavioral analysis software. Results were compared to the
1432 behavior seen in response to catnip (**Figure 7A**). The Kruskal-Wallis test was used to test for differences.
1433 P values shown are from Dunn's post-hoc test. * P < 0.05; ** P < 0.01



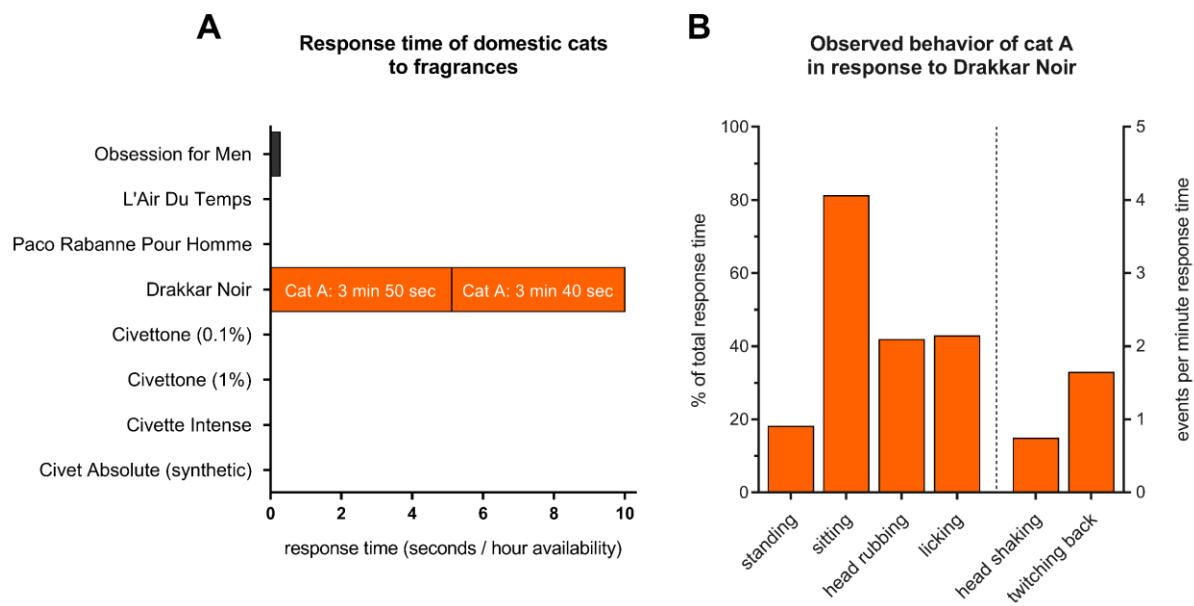
1434

1435 **Figure 17. Heatmaps showing similarities and differences in body position and behaviors of 4 cats**
1436 **in response to cat-attracting plants and single compounds.** Not all cats responded to all classes of
1437 single compounds and therefore comparisons differ between cats. Responses to actinidine and
1438 dihydroactinidiolide of cat O and to dihydroactinidiolide of cat Z are shown but were not included in the
1439 statistical analysis because the number of responses were two or less. Unless otherwise indicated,
1440 numbers represent the median. The Kruskal-Wallis test followed by Dunn's post-hoc test or the Mann
1441 Whitney test was done to test for statistically significant differences. #, frequency; s, seconds; ns, not
1442 statistically significantly different; * P < 0.05, ** P < 0.01, *** P < 0.001



1443

1444 **Figure 18. Comparison of behavior between responses to *N. cataria*, nepetalactone and**
1445 **dihydroactinidiolide.** Results from behavioral analysis in BORIS of responses of cat V to *N. cataria*
1446 (n=9), *cis-trans*-nepetalactone (n=4) and dihydroactinidiolide (n=5) are shown. Some of the responses
1447 were short and this may have contributed to some outliers. Head shaking and rolling on the side are
1448 plotted on the right Y axis. There were no significant differences in behavior between catnip,
1449 nepetalactone and dihydroactinidiolide (Kruskal-Wallis and Dunn's post-hoc test).



1450

1451 **Figure 19. Responses of five domestic cats (*Felis catus*) to fragrances attractive to *Panthera***
1452 **(jaguar, leopard, snow leopard, lion and tiger) and *Acinonyx jubatus* (cheetah).** (A) Response
1453 duration plotted as time per hour the fragrances were available to the cats, with the response time of two
1454 responses by cat A to Drakkar Noir shown within the bar. Obsession for Men was available for 75 hours
1455 (5 days), Drakkar Noir 45 hours (3 days), L'Air Du Temps and Paco Rabanne 30 hours (2 days), and the
1456 other fragrances for 15 hours (1 day). (B) Analysis of the observed behavior of cat A in response to
1457 Drakkar Noir. The average of the two responses is shown. Body position, head rubbing, and licking are
1458 shown as the percentage of the response duration, while head shaking and twitching of the back are
1459 shown as events per minute of response and are plotted on the right Y axis.

	<i>A. indica</i> lyophilized roots	<i>A. polygama</i> dried fruit galls	<i>L. tatarica</i> sawdust wood	<i>N. cataria</i> dried leaves		<i>V. officinalis</i> dried roots
				Frontiers	SmartyKat	
nepetalactone (1)	-	-	-	73.0	35.5	-
epinepetalactone (2)	-	-	-	7.5	5.5	-
dihydronepetalactone (3)	-	20.0	-	8.0	112.0	-
isodihydronepetalactone (4)	6.0	13.5	4.5	11.0	103.0	4.5
<i>trans</i> -dihydronepetalactone	-	7.0	-	-	-	-
<i>trans</i> -isodihydronepetalactone	-	15.5	-	-	6.5	-
neonepetalactone (5)	-	42.0	-	-	-	-
isoneonepetalactone (6)	-	20.0	-	-	-	-
iridomyrmecin (7)	-	5.5	5.0	6.0	5.0	4.0
isoiridomyrmecin (8)	5.5	17.0	14.0	5.0	7.0	8.0
actinidine (9)	56.0	126.5	19.0	14.5	14.0	41.5
dihydroactinidiolide (10)	-	-	-	16.0	18.0	-

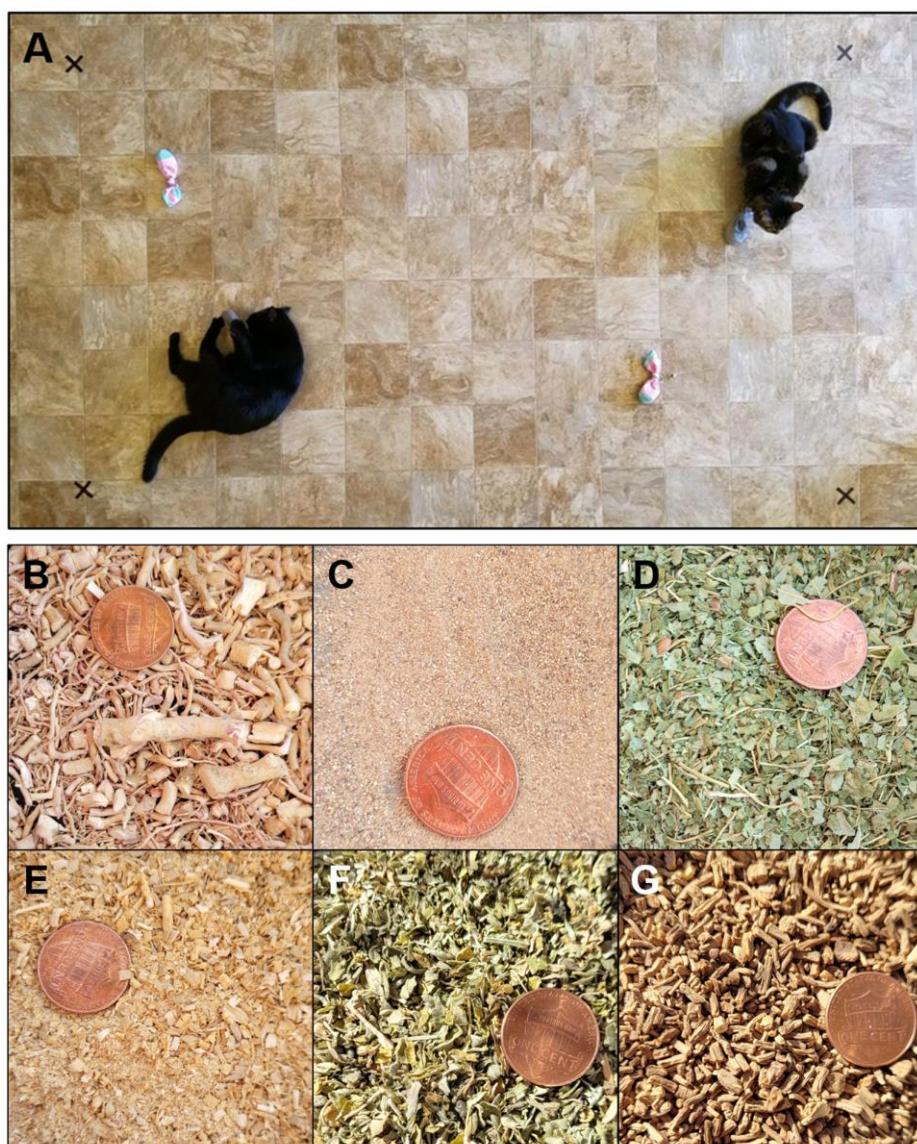
	<i>A. polygama</i>				tinctures			<i>M. trifoliata</i> dried leaves
	dried fruit galls	dried leaves (TX)	dried stem (TX)	dried stem (Asia)	<i>L. tatarica</i>	<i>N. cataria</i>	<i>V. officinalis</i>	
nepetalactone (1)	-	-	-	-	-	2.0	-	-
epinepetalactone (2)	-	-	-	-	-	-	-	-
dihydronepetalactone (3)	20.0	13.5	8.0	7.0	-	5.0	-	-
isodihydronepetalactone (4)	13.5	15.0	-	-	-	5.5	-	-
<i>trans</i> -dihydronepetalactone	7.0	27.5	8.5	8.0	-	-	-	-
<i>trans</i> -isodihydronepetalactone	15.5	37.0	8.5	7.0	-	-	-	-
neonepetalactone (5)	42.0	-	-	21.0	-	-	-	-
isoneonepetalactone (6)	20.0	-	-	-	-	-	-	-
iridomyrmecin (7)	5.5	14.5	-	-	-	-	-	-
isoiridomyrmecin (8)	17.0	14.0	-	-	-	-	2.5	-
actinidine (9)	126.5	392.0	23.0	53.0	2.5	-	22.0	-
dihydroactinidiolide (10)	-	-	15.5	-	-	-	-	49.0

1460

1461 **Figure 20. Quantitation of cat-attracting compounds in plants using GC-MS.** The plant tissues used
1462 for this analysis were fresh samples. They were taken from the same bags of plant material that were
1463 used for the 10 x 10-hour testing. Amounts are reported as μg per gram plant material, except for the
1464 tinctures ($\mu\text{g/mL}$ tincture). Tinctures were made by adding 5 volumes ethanol (500 ml) to one volume of
1465 plant tissue (10, 20 and 50 grams for catnip, Tatarian honeysuckle and valerian root, respectively).
1466 Dashes indicate that the compound was not detected. Numbers are rounded to the nearest half. Reported
1467 values are the average of three separate extractions of the plant material. Unrounded numbers with
1468 standard error of the mean are shown in **Supplementary Figure 12**. Where compounds (3) and (4) are
1469 reduced forms of compound (1), *trans*-dihydronepetalactone and *trans*-isodihydronepetalactone are
1470 reduced forms of compound (2). *Trans*-dihydronepetalactone and *trans*-isodihydronepetalactone were not
1471 used in the bioassays with domestic cats.

1472 **Supplementary figures**

1473



1474

1475 **Supplementary Figure 1.** Top (A): The testing area with 4 mounted socks. The black x's served to
1476 assure the relevant area of the testing area was being captured by the camera. Bottom: Close-up
1477 photographs of the plant materials used in the study: (B) lyophilized and cut *Acalypha indica* or Indian
1478 nettle root, (C) dried, powdered *Actinidia polygama* or silver vine fruit gall, (D) dried and cut Texas-grown
1479 *A. polygama* leaves, (E) *Lonicera tatarica* or Tatarian honeysuckle sawdust, (F) dried and cut *Nepeta*
1480 *cataria* or catnip leaves, and (G) dried and cut *Valeriana officinalis* or valerian root. A United States penny
1481 (19 mm diameter) is used as a size reference.

A

Plant	Dates tested	Response testing										M	T	W	T	F	S	S
		1	2	3	4	5	6	7	8	9	10							
<i>L. tatarica</i>	12 Jun - 13 Jul 2018	1	2	3	4	5												
<i>A. polygama</i>	19 Jul - 16 Aug 2018		1	2	3	4	5					6	7	8			9	10
<i>A. indica</i>	23 Aug - 20 Sep 2018		1	2	3	4	5					6	7	8			9	10
<i>N. cataria</i>	1 - 29 Nov 2018		1	2	3	4	5					6	7	8			9	10
<i>V. officinalis</i>	28 Mar - 25 Apr 2019		1	2	3	4	5					6	7	8			9	10

week 1 week 2 interstimulus interval week 4 week 5

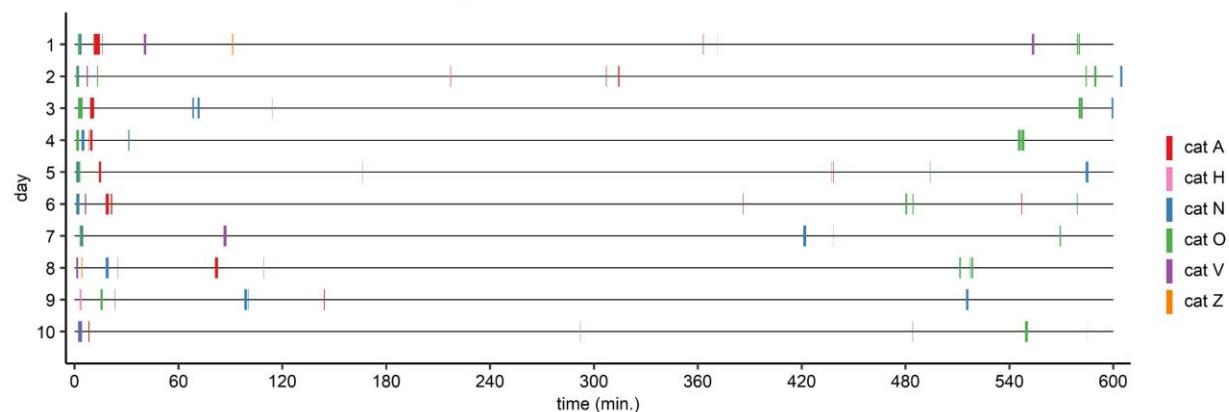
B

Plant	Dates tested	Habituation / dishabituation testing												Amount (g)	Hours available		
		1	2	3	4	5	6	7	8	9	10	11	12				
<i>A. polygama</i>	16 - 27 Jul 2019	1	2	3	4	5	6	7	8	9	10	11	12	0.5	5	5	2 (20:00 - 22:00)
<i>L. tatarica</i>	26 Aug - 5 Sep 2019	1	2	3	4	5	6	7	8	9	10	11		2.5	2.5		2 (20:00 - 22:00)
<i>N. cataria</i>	18 - 28 Sep 2019	1	2	3	4	5	6	7	8	9	10	11		2.5	2.5		2 (20:00 - 22:00)
<i>A. polygama</i>	20 - 31 Dec 2019	1	2	3	4	5	6	7	8	9	10	11	12	2.5	2.5	2.5	12 (10:00 - 22:00)

1482

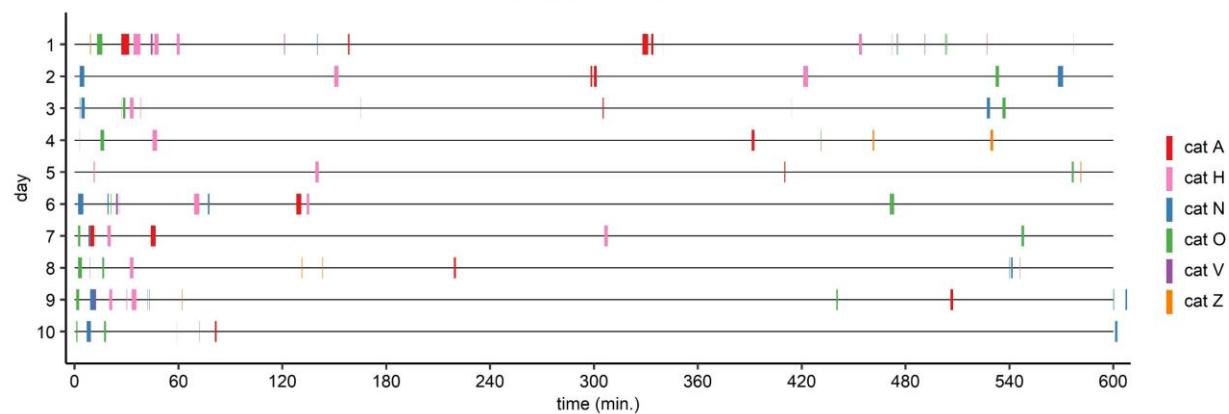
1483 **Supplementary Figure 2.** Timeline for testing cat-attracting plants. **(A)** Each cat-attracting plant was
 1484 tested on 10 different days (no. 1 – 10), 10 hours per day, to learn more about response duration,
 1485 response frequency, and behavior during the responses. The tests were done in two periods of two
 1486 weeks, separated by an interstimulus interval of at least 9 days. Testing was done 2 – 3 days per week.
 1487 There was always at least one week between testing the different plants (see “Dates tested”). **(B)** Three
 1488 different cat-attracting plants were offered for 10 consecutive days (2 or 12 hours per day) to test for
 1489 habituation. After 10 days the cats were offered a different plant to test dishabituation. Plant materials are
 1490 color-coded according to the color scheme used in **(A)**.

Acalypha indica (Indian nettle)



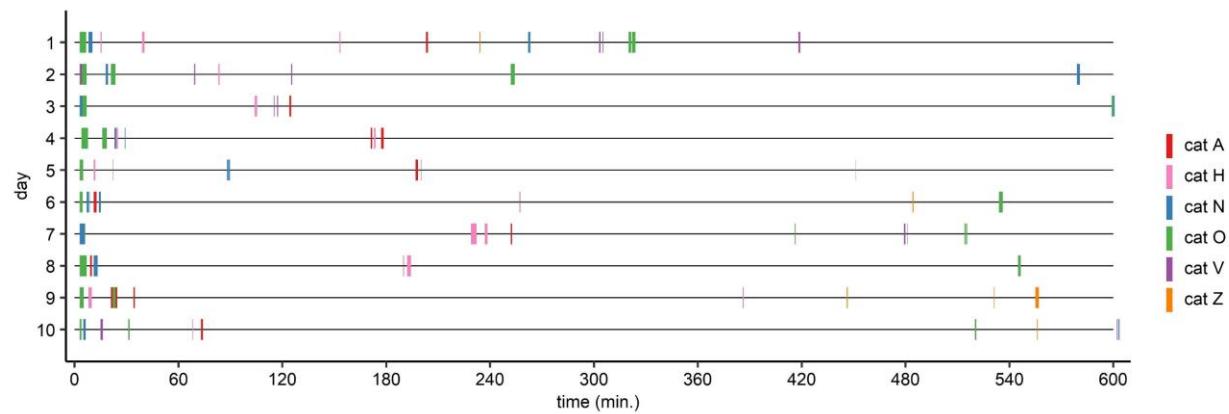
1491

Actinidia polygama (silver vine)

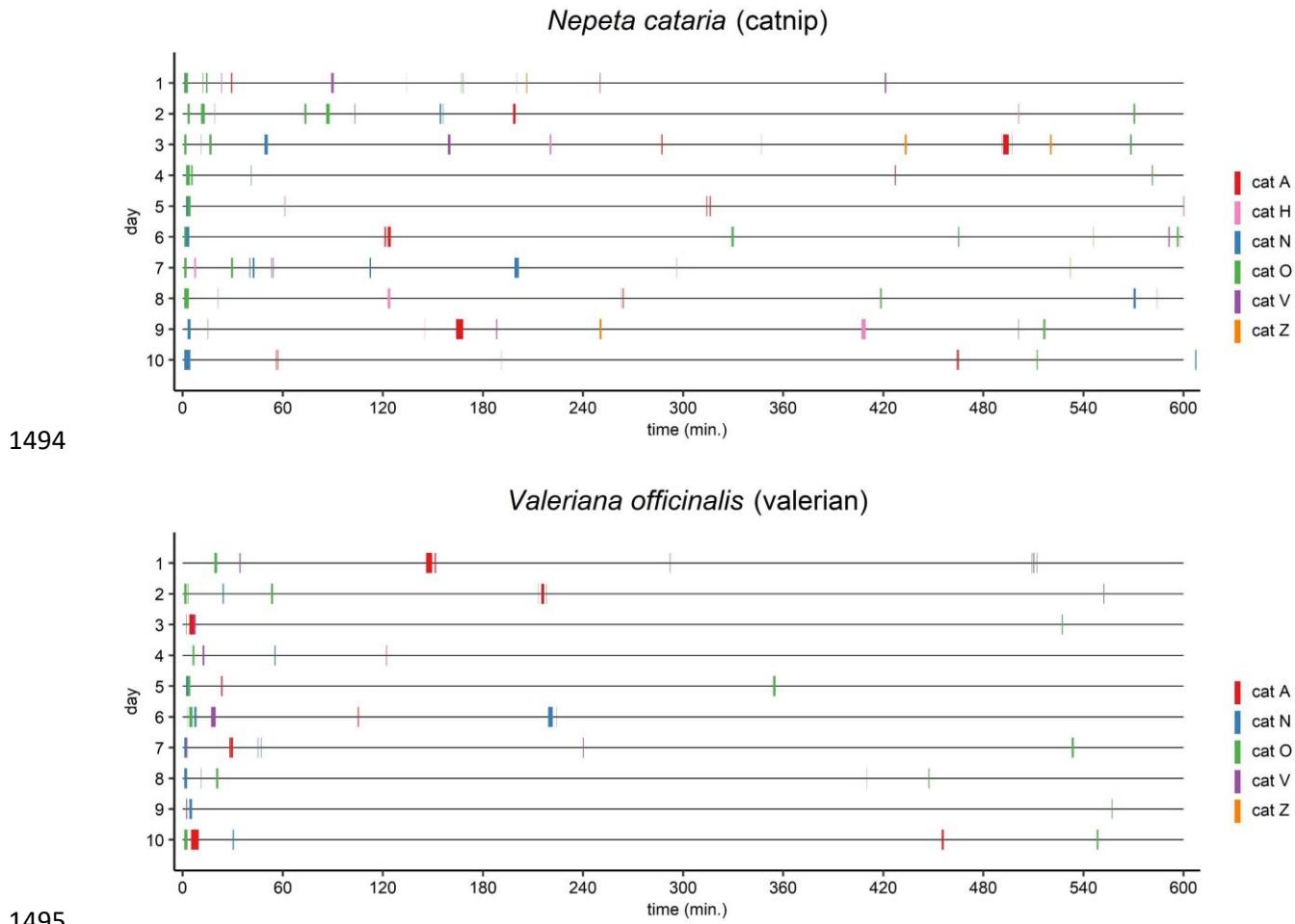


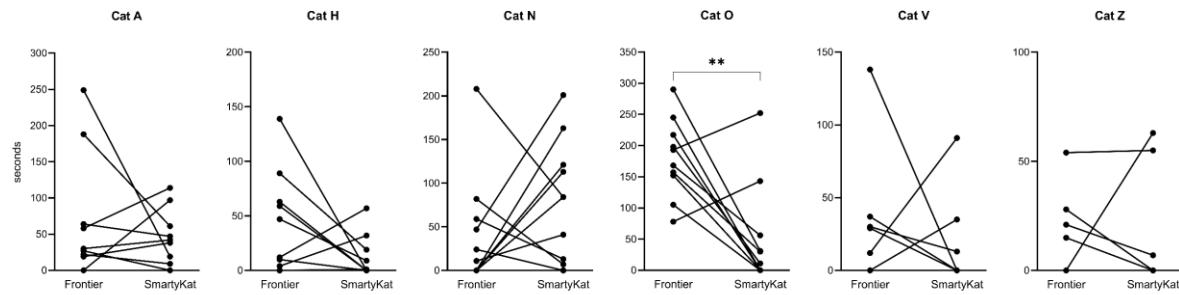
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Lonicera tatarica (Tatarian honeysuckle)



1493





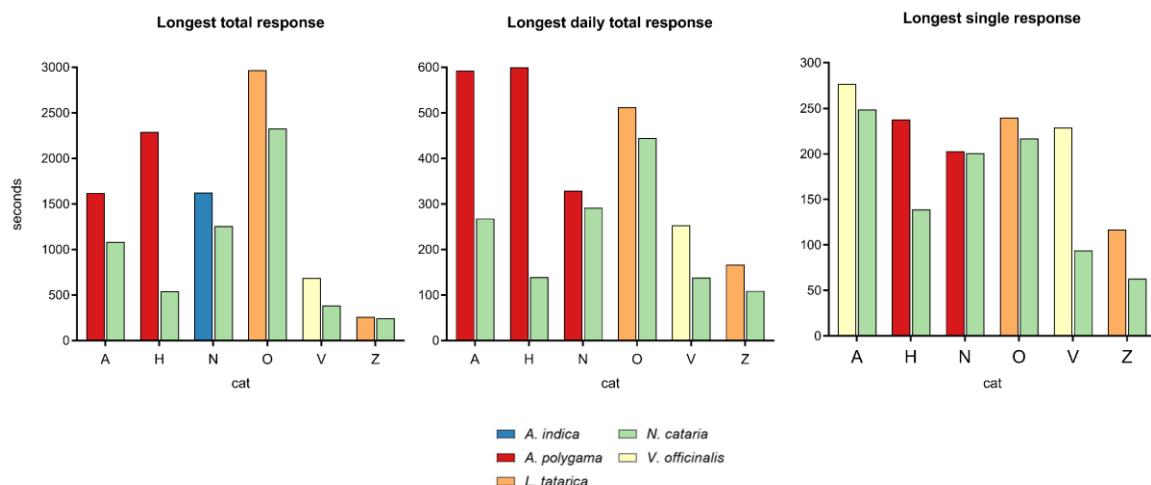
1500

1501 **Supplementary Figure 4.** Response duration of 6 domestic cats to two different sources of *N. cataria*.

1502 Each dot represents the total response duration on one of the 10 testing days. The median response

1503 duration of cat O to catnip from Frontier was significantly longer than the median response duration to

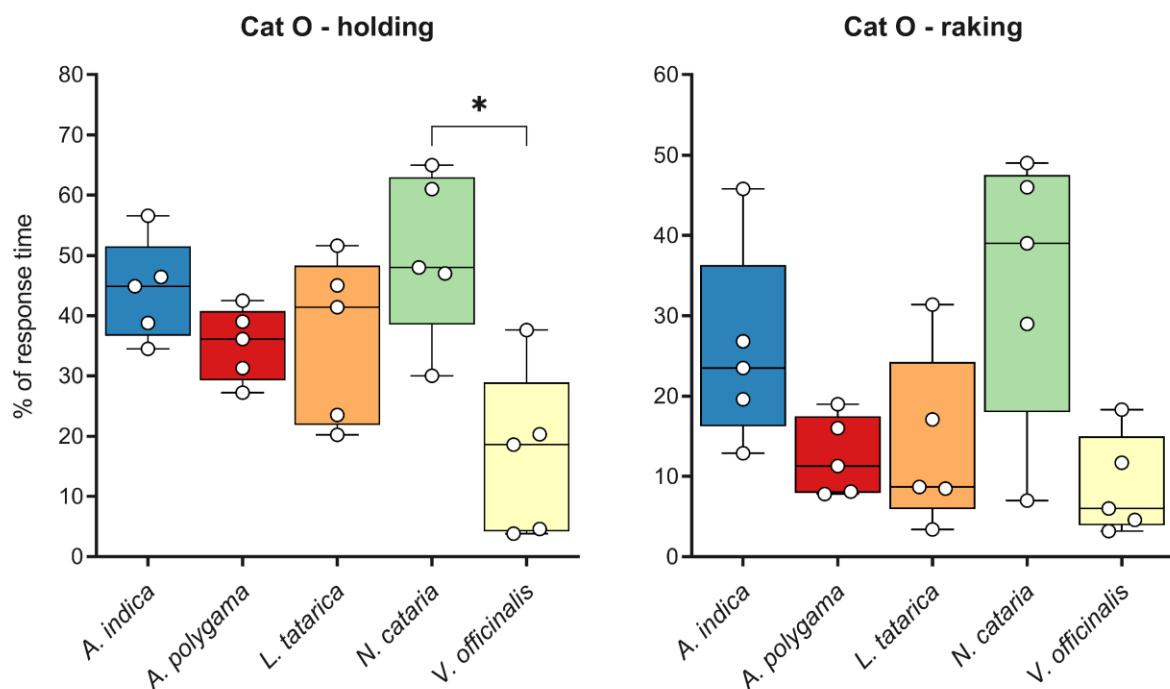
1504 catnip from SmartyKat ($P = 0.0098$, Wilcoxon matched-pairs signed rank test). ** $P < 0.01$



1505

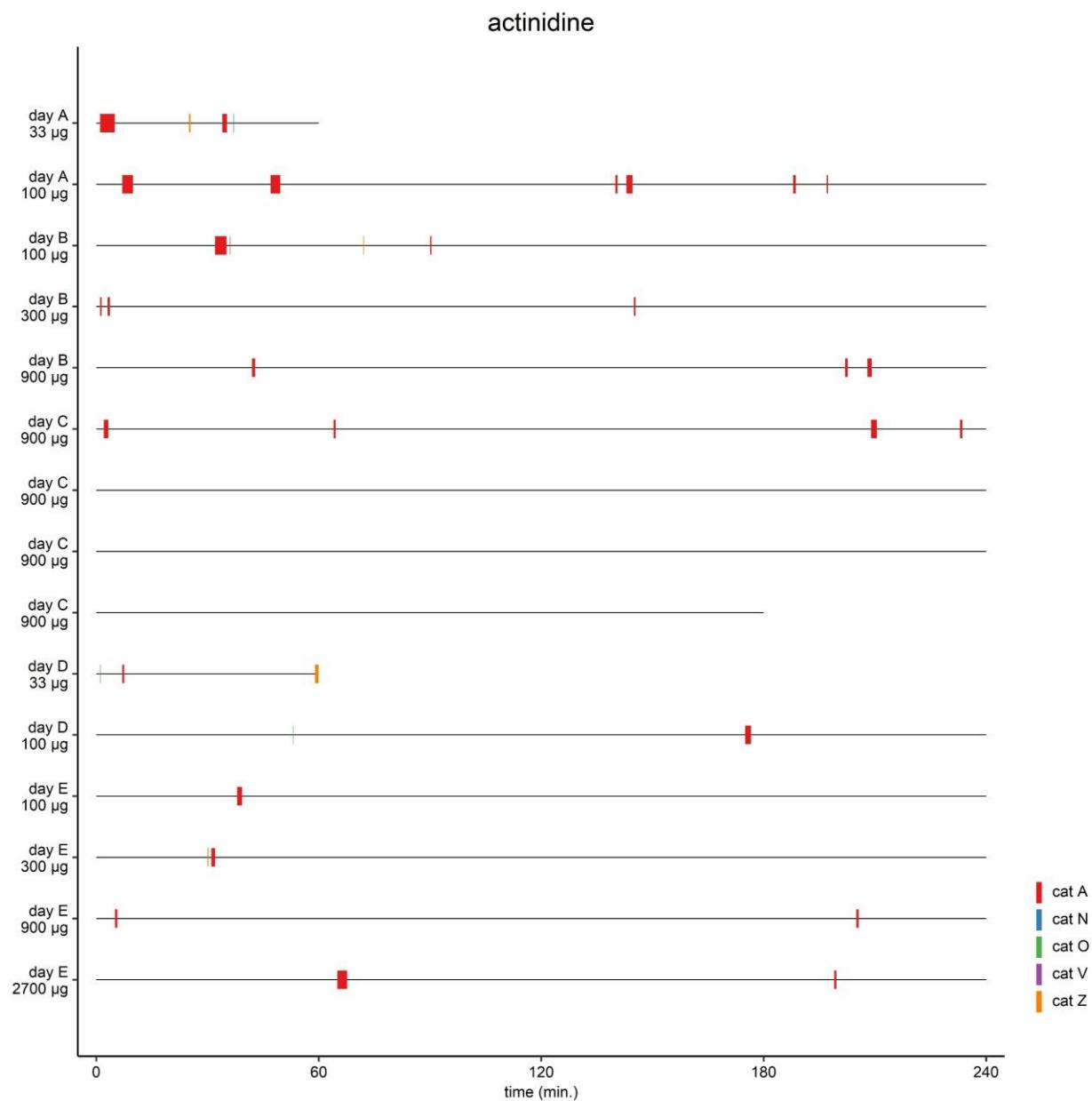
1506 **Supplementary Figure 5.** The longest total response (100 hours availability), the longest daily response

1507 (10 hours availability), and the longest single response for each cat, compared to *N. cataria* (catnip).

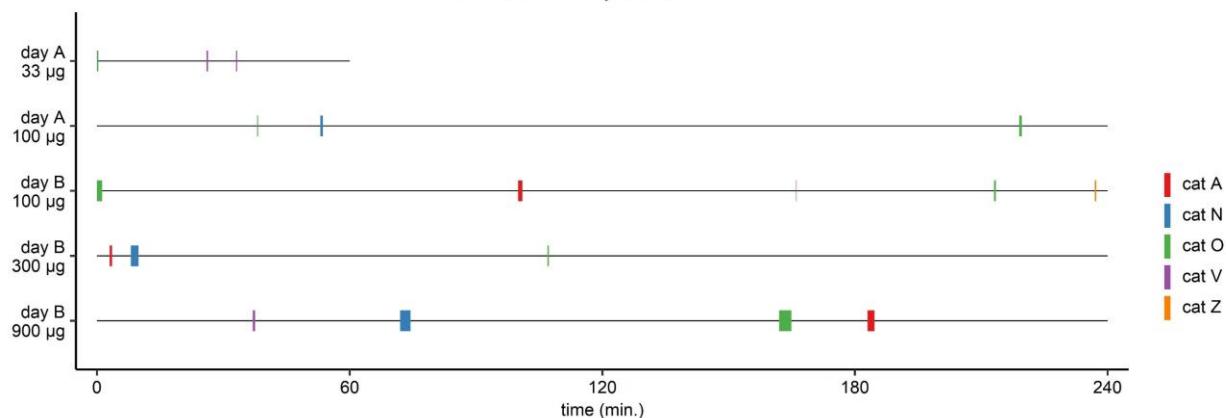


1508

1509 **Supplementary Figure 6.** Box and whisker plots showing the time spent holding and raking by cat O in
1510 response to 5 cat-attracting plant species. Data from 5 responses nearest to 60 seconds are shown for
1511 each plant. Time is expressed as the percentage of the total response duration. This is a different
1512 representation of the data shown in **Figure 7C**. The differences between the plants for both holding and
1513 raking were statistically significant (Kruskal-Wallis, $P < 0.05$). The P value shown is from Dunn's post-hoc
1514 test. * $P < 0.05$

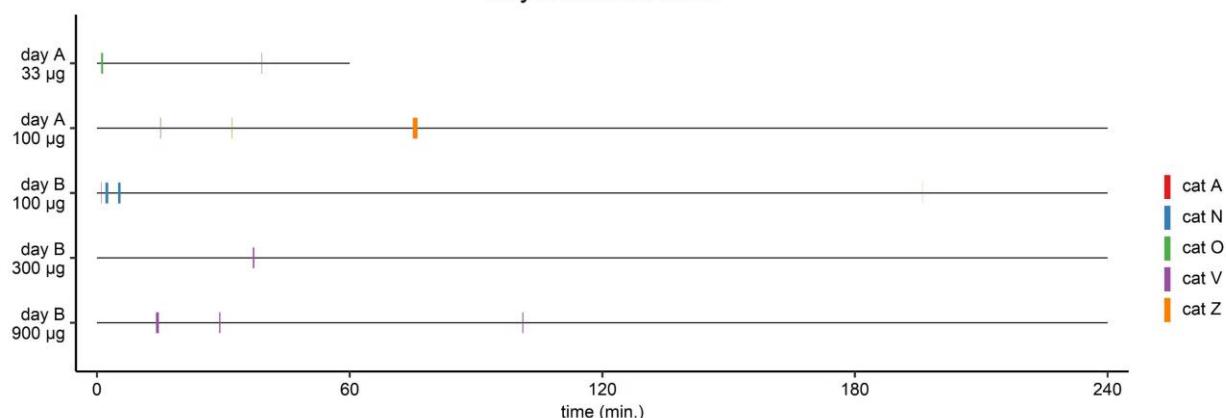


cis-trans-nepetalactone



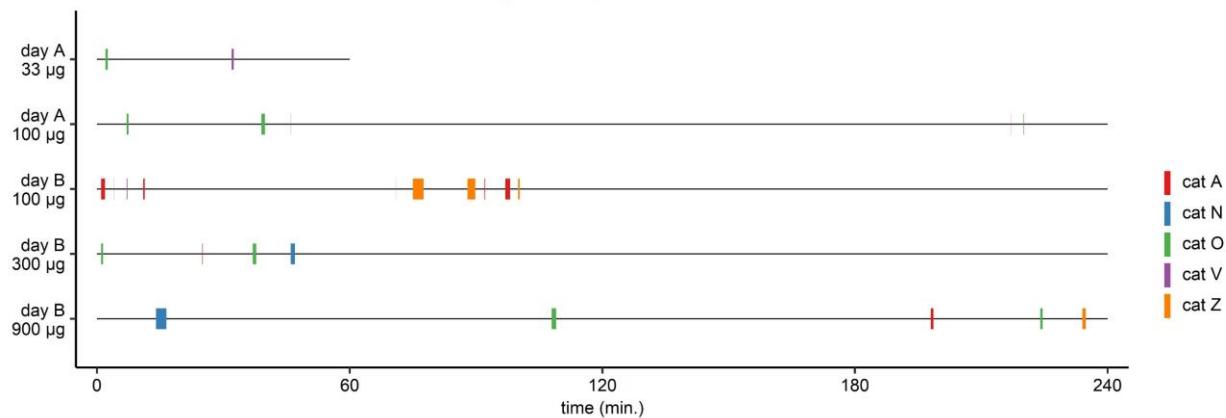
1516

dihydroactinidiolide



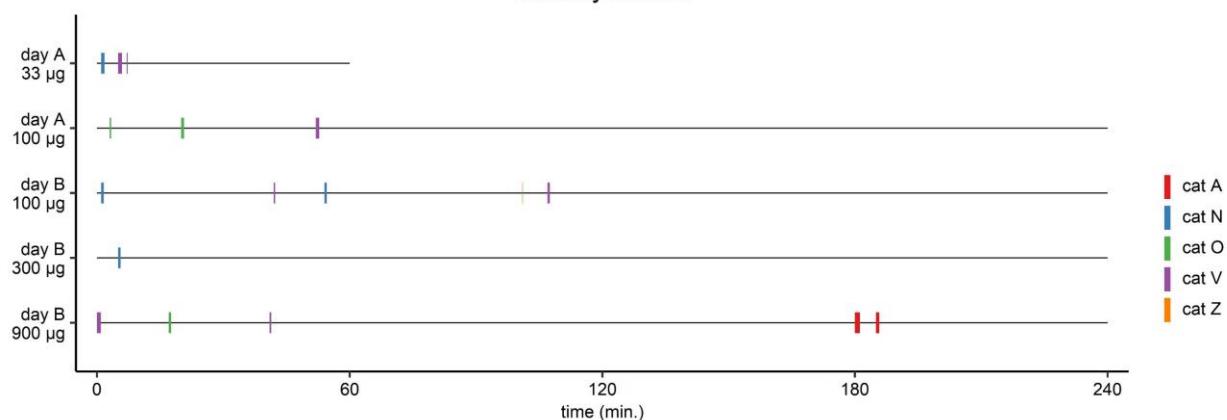
1517

dihydronepetalactone



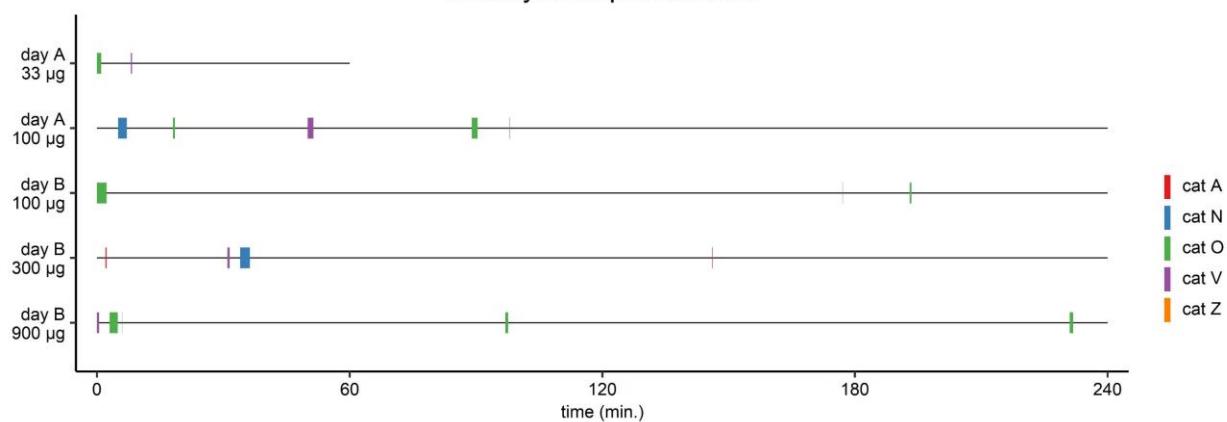
1518

iridomyrmecin



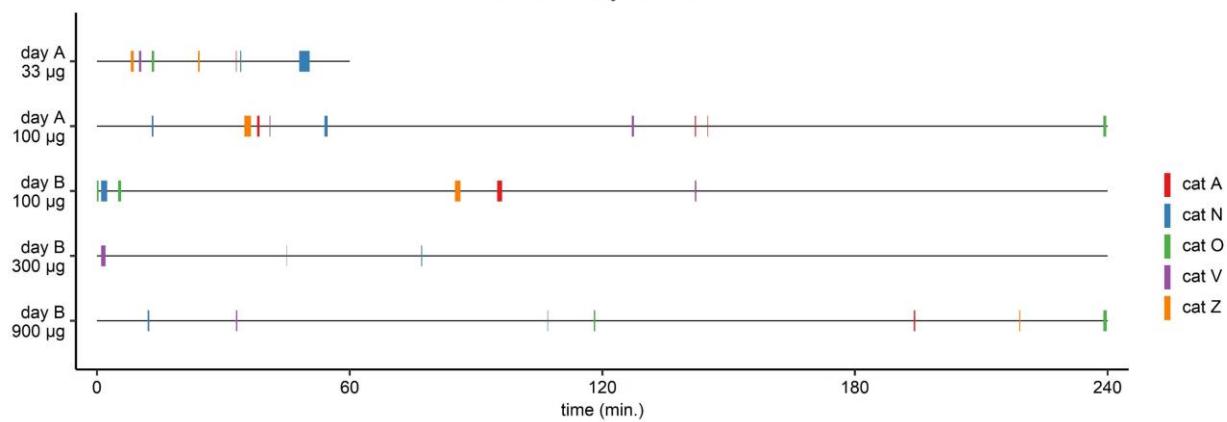
1519

isodihydronpetalactone



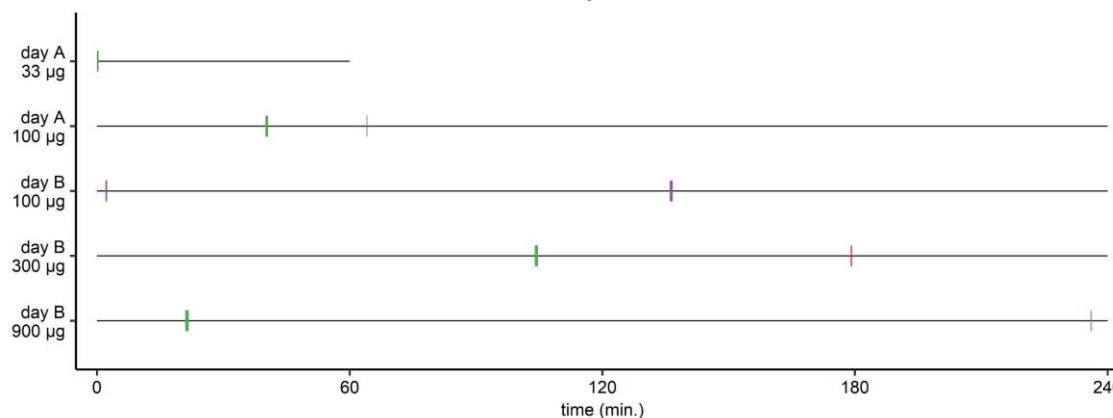
1520

isoiridomyrmecin



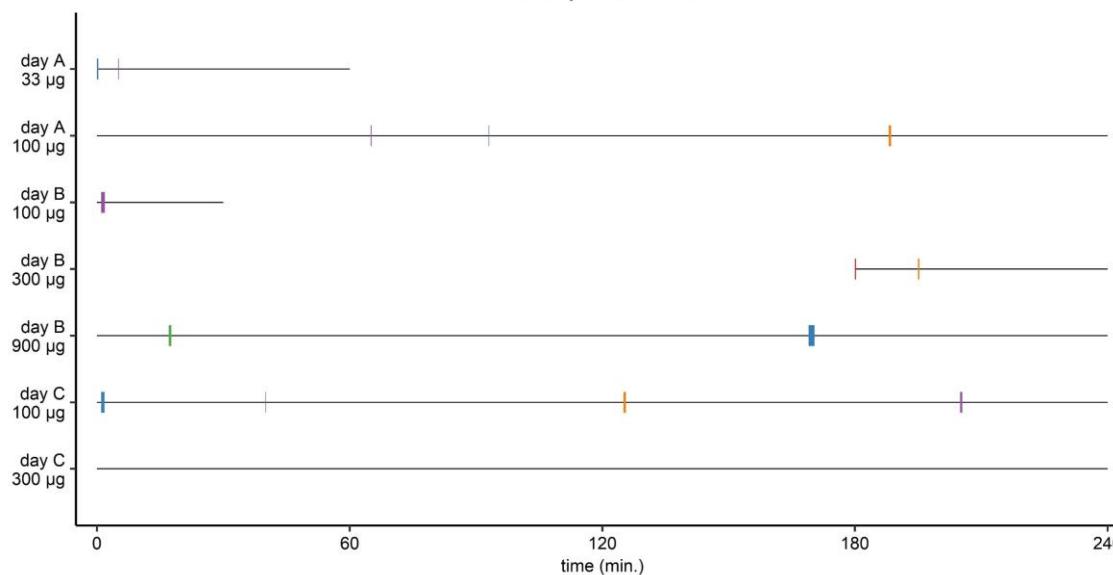
1521

isoneonepetalactone

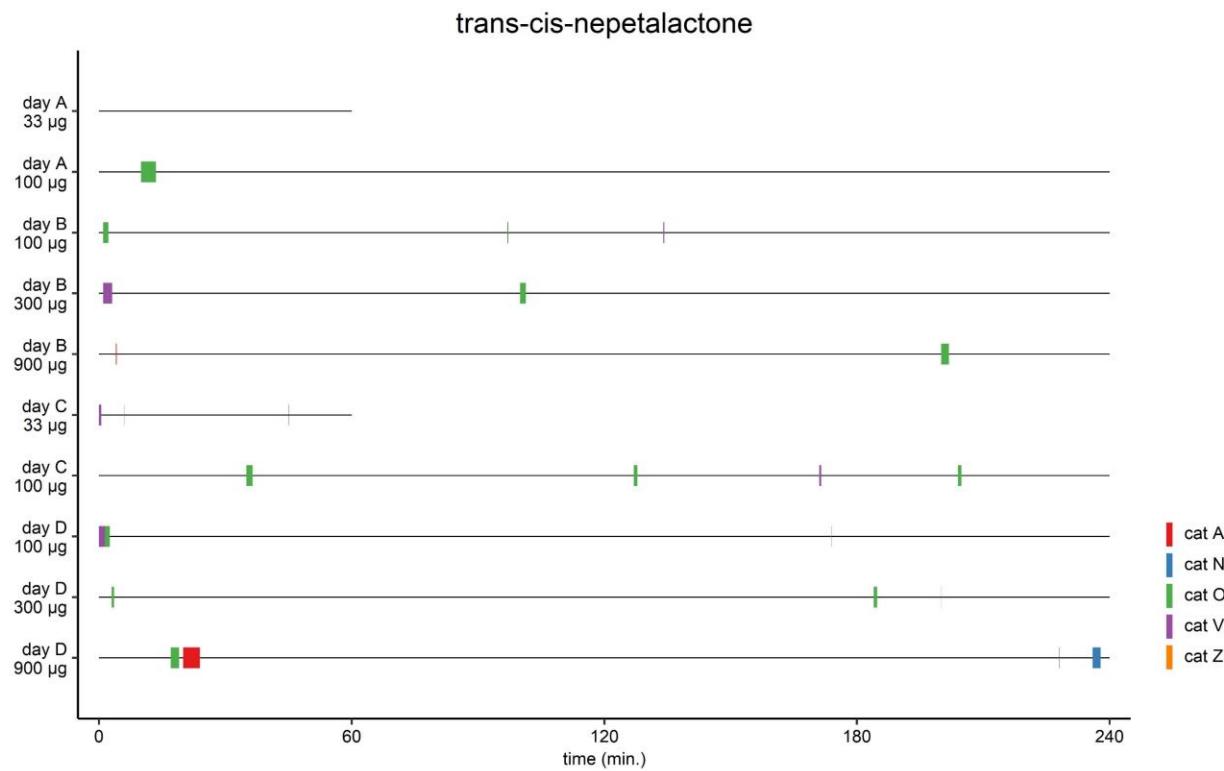


1522

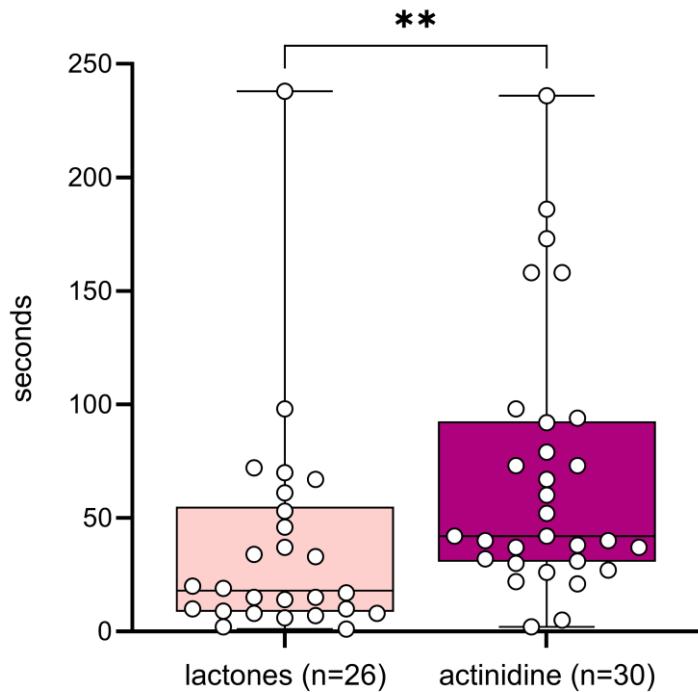
neonepetalactone



1523

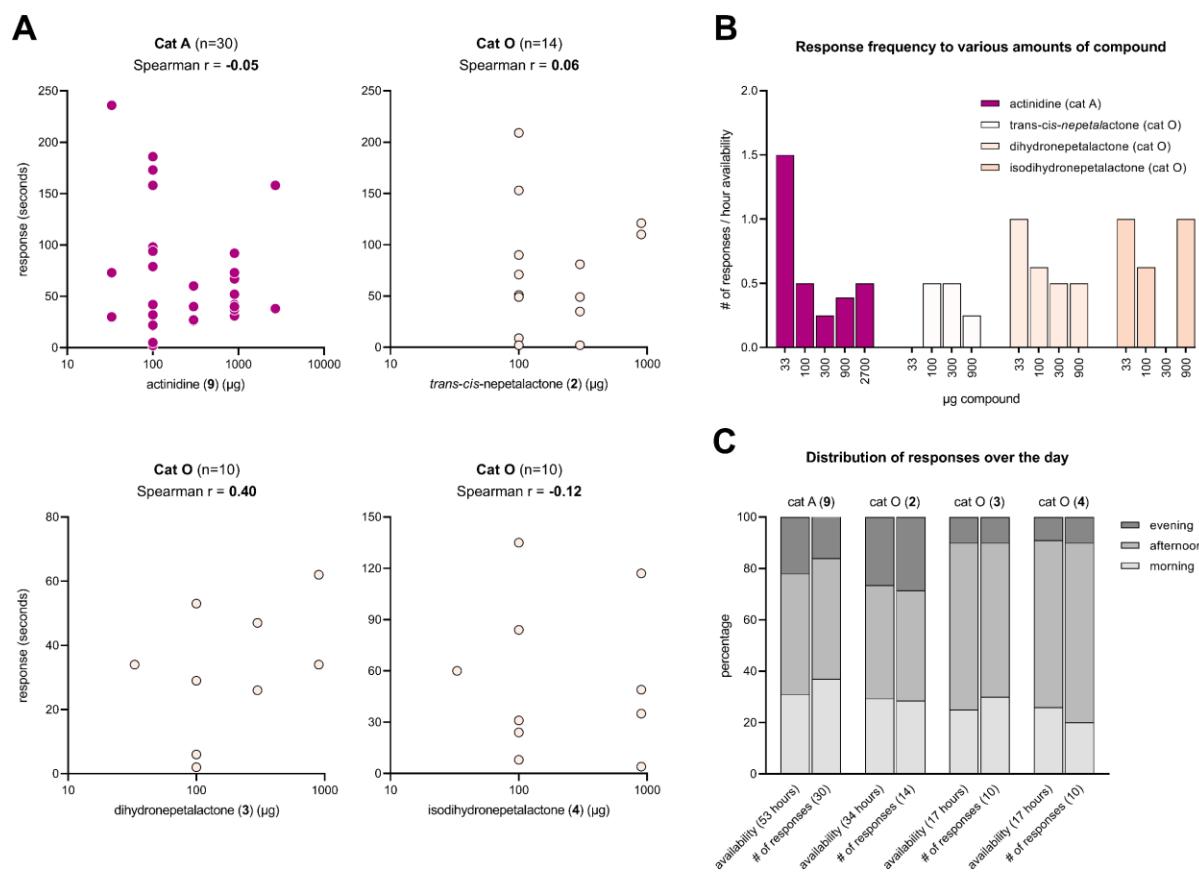


Response duration of cat A to iridoids



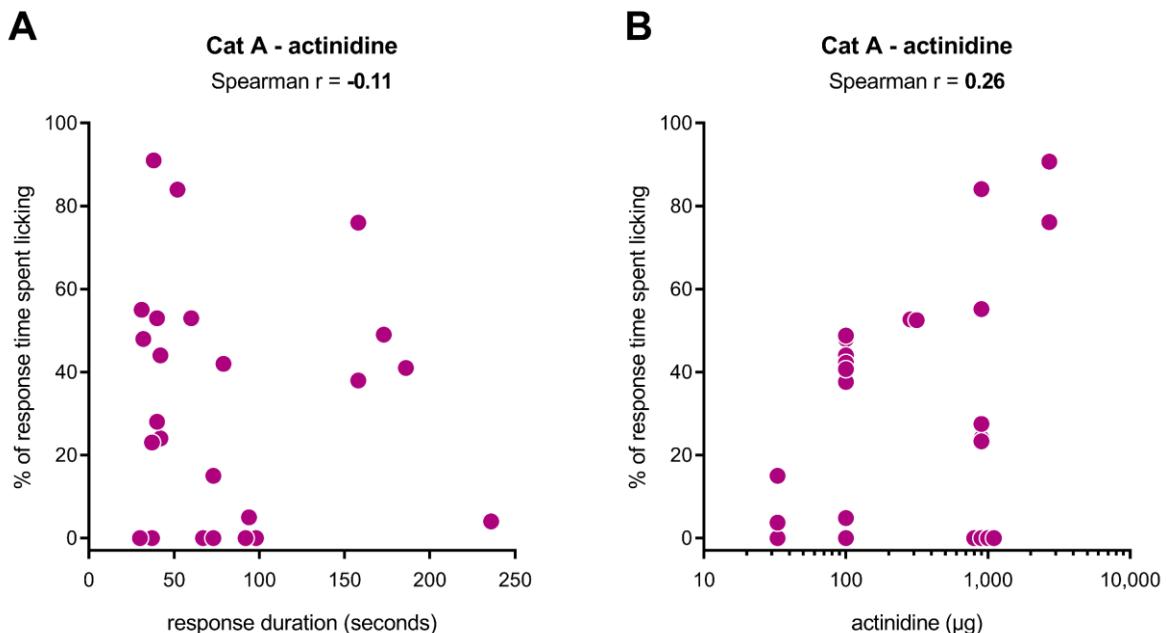
1533

1534 **Supplementary Figure 8.** Duration of individual responses of cat A to the lactones and actinidine. Each
1535 individual response is shown as a dot. The difference in response duration between the lactones and
1536 actinidine is statistically significant (Mann-Whitney test). ** P < 0.01



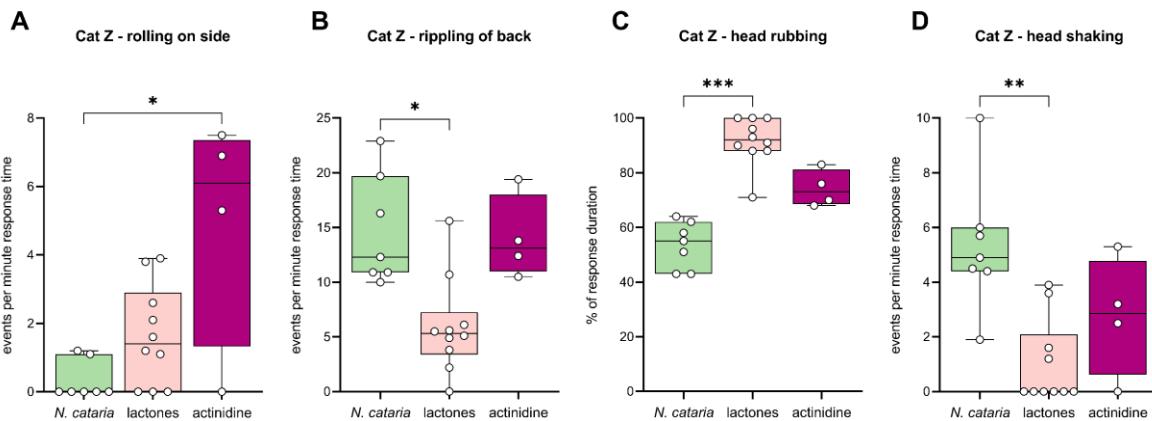
1537

1538 **Supplementary Figure 9.** Data from compounds for which at least 10 responses from an individual cat
 1539 were observed were used to study correlation between response duration/frequency and the amount of
 1540 compound used, as well between response frequency and time of the day. **(A)** Response time to single
 1541 compounds plotted against the various quantities of the compounds used in the tests: 33, 100, 300, 900
 1542 and 2700 μ g. The quantities are shown on a log10 scale. **(B)** Response frequency per hour availability
 1543 shown for the different quantities of single compounds tested. **(C)** Distribution of responses over the day
 1544 (morning, afternoon and evening) compared to the distribution the olfactory stimuli were available to the
 1545 cat (morning, afternoon and evening). Both the number of responses and the time each compound was
 1546 available to the cat are expressed as a percentage. The total number of responses and hours availability
 1547 are shown between parentheses. Bold numbers refer to the compound (**Table 3**). The Fisher exact test
 1548 was used to test for differences in distribution (all P values > 0.05).



1549

1550 **Supplementary Figure 10.** Absence of correlation between time spent licking and response duration (**A**)
1551 and time spent licking and actinidine quantity (33, 100, 300, 900 and 2700 μ g) (**B**). Data from 24
1552 responses longer than 30 seconds in duration to actinidine of cat A are shown. Some data points were
1553 overlapping and were edited for visualization purposes only. Actinidine quantity (μ g) is shown on a log10
1554 scale.



1555

1556 **Supplementary Figure 11.** Differences in behavior of cat Z between responses to *N. cataria*, lactones
1557 and actinidine.

Plant material after bioassays

	<i>A. indica</i> lyophilized roots	<i>A. polygama</i> dried fruit galls	<i>L. tatarica</i> sawdust wood	<i>N. cataria</i> dried leaves		<i>V. officinalis</i> dried roots
				Frontiers	SmartyKat	
nepetalactone (1)	-	-	-	40.5 (±1.1)	38.5 (±1.7)	-
epinepetalactone (2)	-	-	-	1.8 (±1.8)	5.7 (±0.1)	-
dihydronepetalactone (3)	-	14.1 (±0.7)	-	17.9 (±0.1)	89.0 (±1.0)	-
isodihydronepetalactone (4)	5.1 (±0.1)	7.1 (±0.1)	4.5 (±0.1)	19.3 (±0.1)	68.5 (±1.2)	2.9 (±1.5)
<i>trans</i> -dihydronepetalactone	-	6.1 (±0.1)	-	-	-	-
<i>trans</i> -isodihydronepetalactone	-	12.2 (±0.2)	-	-	6.2 (±0.1)	-
neonepetalactone (5)	-	30.1 (±2.5)	-	-	-	-
isoneonepetalactone (6)	-	16.4 (±1.3)	-	-	-	-
iridomyrmecin (7)	-	4.4 (±0.1)	4.2 (±0.1)	5.6 (±0.1)	5.0 (±0.1)	3.9 (±0.1)
isoiridomyrmecin (8)	4.1 (±0.1)	8.7 (±1.1)	8.3 (±0.1)	4.5 (±0.2)	6.5 (±0.1)	7.0 (±0.1)
actinidine (9)	41.8 (±1.7)	104.1 (±3.4)	43.9 (±0.1)	14.2 (±0.1)	14.3 (±0.1)	36.6 (±1.0)
dihydroactinidiolide (10)	-	-	-	15.4 (±0.9)	17.2 (±1.1)	-

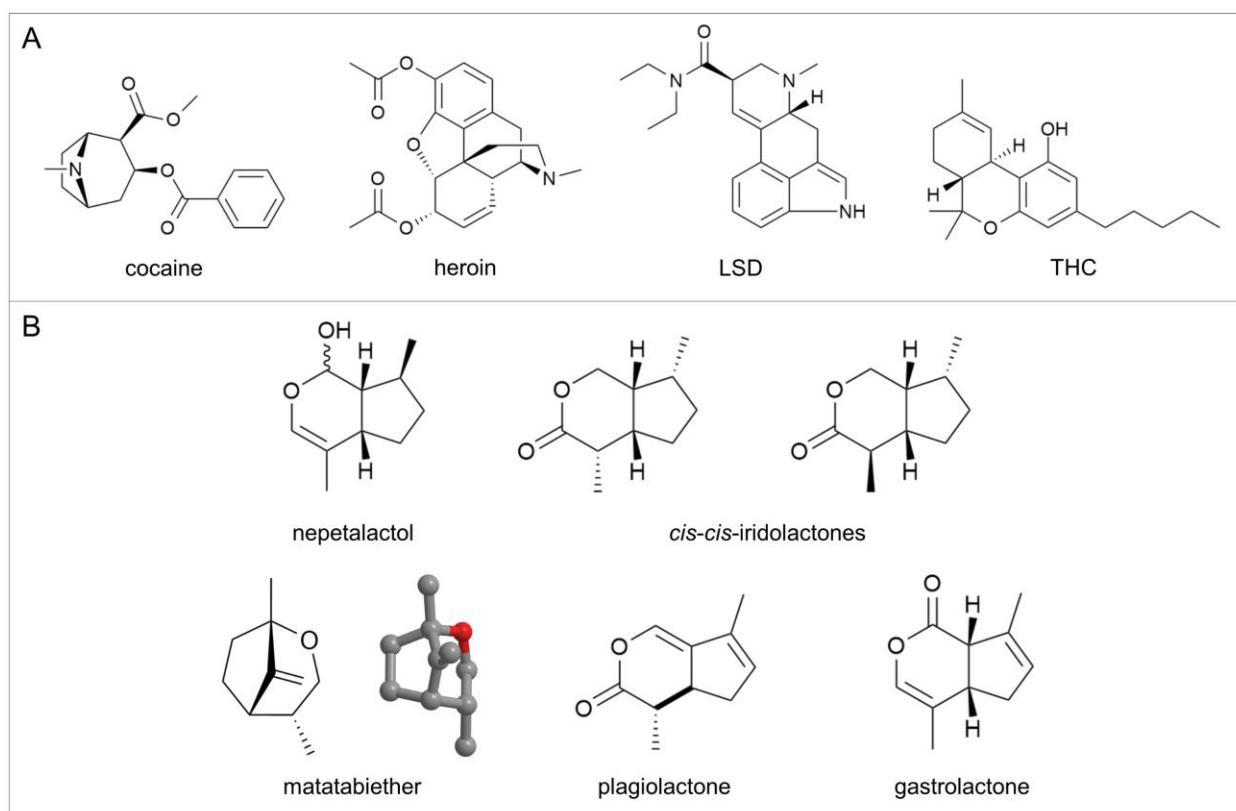
Fresh plant material

	<i>A. indica</i> lyophilized roots	<i>A. polygama</i> dried fruit galls	<i>L. tatarica</i> sawdust wood	<i>N. cataria</i> dried leaves		<i>V. officinalis</i> dried roots
				Frontiers	SmartyKat	
nepetalactone (1)	-	-	-	73.0 (±4.3)	35.5 (±0.6)	-
epinepetalactone (2)	-	-	-	7.3 (±0.1)	5.5 (±0.1)	-
dihydronepetalactone (3)	-	20.2 (±0.7)	-	8.1 (±0.3)	112.1 (±0.8)	-
isodihydronepetalactone (4)	6.1 (±0.1)	13.5 (±0.1)	4.6 (±0.1)	10.8 (±0.2)	102.9 (±0.9)	4.5 (±0.1)
<i>trans</i> -dihydronepetalactone	-	7.0 (±0.1)	-	-	-	-
<i>trans</i> -isodihydronepetalactone	-	15.3 (±0.2)	-	-	6.5 (±0.1)	-
neonepetalactone (5)	-	42.2 (±0.4)	-	-	-	-
isoneonepetalactone (6)	-	20.1 (±0.2)	-	-	-	-
iridomyrmecin (7)	-	5.3 (±0.1)	4.9 (±0.5)	6.2 (±0.1)	5.0 (±0.1)	4.2 (±0.1)
isoiridomyrmecin (8)	5.5 (±0.2)	17.1 (±1.9)	13.8 (±2.1)	4.9 (±0.1)	6.9 (±0.2)	8.2 (±0.3)
actinidine (9)	56.0 (±1.7)	126.3 (±1.3)	19.1 (±0.6)	14.4 (±0.3)	14.2 (±0.1)	41.4 (±0.8)
dihydroactinidiolide (10)	-	-	-	15.9 (±0.2)	17.9 (±1.4)	-

	<i>A. polygama</i>				tinctures			<i>M. trifoliata</i> dried leaves
	dried fruit galls	dried leaves (TX)	dried stem (TX)	dried stem (Asia)	<i>L. tatarica</i>	<i>N. cataria</i>	<i>V. officinalis</i>	
nepetalactone (1)	-	-	-	-	-	2.2	-	-
epinepetalactone (2)	-	-	-	-	-	-	-	-
dihydronepetalactone (3)	20.2 (±0.7)	13.4 (±0.4)	8.1 (±0.7)	7.2 (±0.2)	-	5.0	-	-
isodihydronepetalactone (4)	13.5 (±0.1)	15.1 (±0.4)	-	-	-	5.4	-	-
<i>trans</i> -dihydronepetalactone	7.0 (±0.1)	27.5 (±0.3)	8.7 (±0.4)	7.9 (±0.7)	-	-	-	-
<i>trans</i> -isodihydronepetalactone	15.3 (±0.2)	37.1 (±1.0)	8.7 (±0.7)	7.1 (±0.5)	-	-	-	-
neonepetalactone (5)	42.2 (±0.4)	-	-	21.2 (±1.3)	-	-	-	-
isoneonepetalactone (6)	20.1 (±0.2)	-	-	-	-	-	-	-
iridomyrmecin (7)	5.3 (±0.1)	14.6 (±0.5)	-	-	-	-	-	-
isoiridomyrmecin (8)	17.1 (±1.9)	14.1 (±0.4)	-	-	-	-	2.3	-
actinidine (9)	126.3 (±1.3)	392.1 (±17.3)	22.8 (±0.2)	53.2 (±1.9)	2.3	-	22.0	-
dihydroactinidiolide (10)	-	-	15.6 (±0.3)	-	-	-	-	49.2 (±0.1)

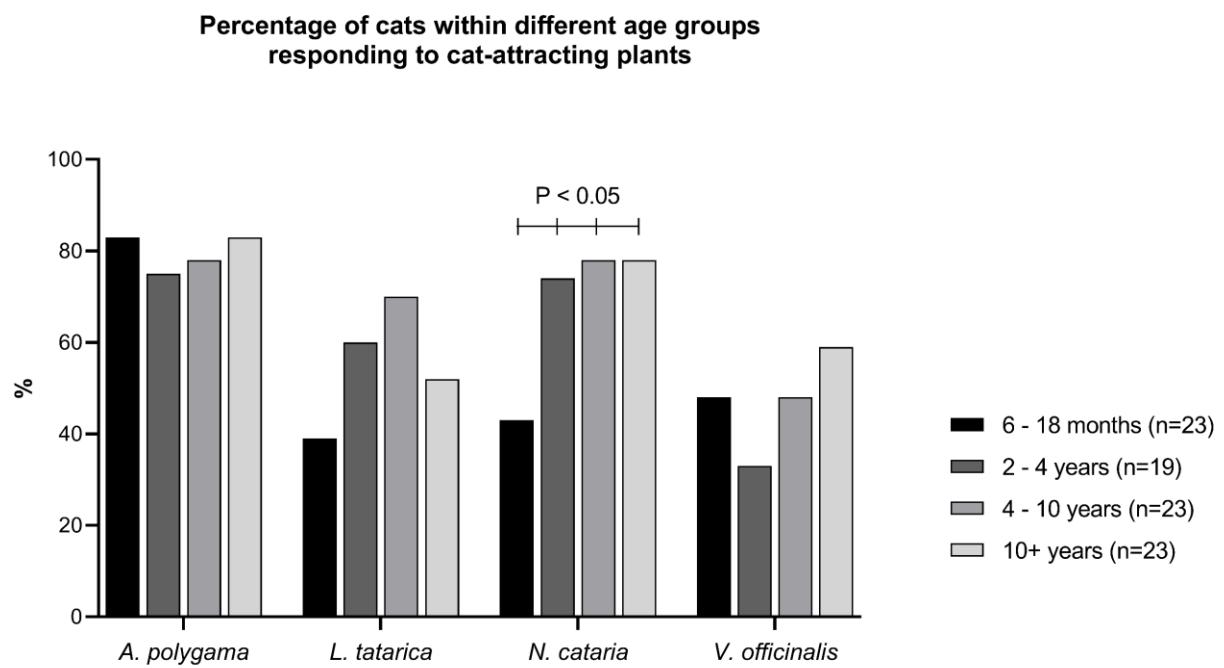
1558

1559 **Supplementary Figure 12. (top)** Quantitation of cat-attracting compounds in the plant material taken
 1560 from the socks after testing 10 × 10 hours in a 5-week testing period. Amounts are reported as µg per
 1561 gram plant material used in this study. Reported values are the average of three separate extractions of
 1562 the plant material. The standard error of the mean is reported between parentheses. Dashes indicate that
 1563 the compound was not detected. **(middle and bottom)** Data from **Figure 20** reported with the standard
 1564 error of the mean and unrounded numbers.



1565

1566 **Supplementary Figure 13. (A)** The structures of cocaine, heroin, LSD and THC do not resemble the
1567 structure of the cat-attracting single compounds (**Figure 12**). **(B)** The structures of nepetalactol, both *cis*-
1568 *cis*-iridolactones, plagiolactone, gastrolactone, and both the 2D and 3D structure of the bridged bicyclic
1569 matatabiether.



1570

1571 **Supplementary Figure 14.** The fraction of domestic cats, within 4 age groups, that responded to a
1572 variety of cat-attracting plants. Significantly fewer of the younger cats (6 – 18 months) showed a response
1573 to catnip when compared to the 3 older groups (43% versus 74, 78 and 78%; P < 0.05, Fisher's exact
1574 test). There were no cats 18 – 24 months of age.

1575 **Supplementary files**

1576

1577 **Supplementary File 1.** Video showing behavior of domestic cats seen in response to cat-attracting plants
1578 and single compounds (**Table 4**). Four recordings are shown for each behavior. (MP4)

1579

1580 **Supplementary File 2.** DNA barcoding sequence of *cox1* from *Pseudasphondylia matatabi*, and
1581 sequences of *matK*, *rbcL*, and *psbA – trnH* from *Actinidia* and *Lonicera* species. (TXT)

1582

1583 **Supplementary File 3.** Raw data. This file contains the raw data from the plant, single compound, and
1584 habituation / dishabituation experiments, as well as the BORIS analyses (plants and single compounds).
1585 (XLSX)

1586

1587 **Supplementary File 4.** Video of cats A, H, N, O, V and Z responding to catnip. One response is shown
1588 for each cat. (MP4)

1589 **Tables**

1590

1591 **Table 1. Age, breed, hair-color and pattern, and gender of the cats who participated in the study.**

Name (abbreviation)	Age ¹	Breed	Color / pattern	Gender
Aguereberry (A)	11Y 1M	domestic short-haired	calico	female
Harvey (H)	1Y 4M	domestic short-haired	orange	female
Namibia (N)	3Y 5M	domestic short-haired	grey tabby	female
Olli (O)	3Y 5M	domestic short-haired	black	male
Vlinder (V)	3Y 5M	domestic short-haired	grey tabby	female
Zappa (Z)	6Y 6M	domestic short-haired	tortoiseshell	female

1592 ¹ Age in years (Y) and months (M) at the start of the study

1593 **Table 2. An overview of the plant materials that were used in this study.**

Plant species (common name)	Tissue	Source / brand
<i>Acalypha indica</i> (Indian nettle)	roots (lyophilized, cut)	Christmas Island, Government of Western Australia
<i>Actinidia polygama</i> (silver vine)	fruit galls (dried, powder)	Smack (smack.co.jp)
<i>Actinidia polygama</i> varieties 'Hot Pepper' (female) and 'Pavel' (male)	leaves (dried, cut) and stem (lignified, dried)	One Green World (Portland, Oregon, USA)
<i>Lonicera tatarica</i> (Tatarian honeysuckle)	wood (sawdust)	The Cat House (Calgary, Alberta, Canada)
<i>Nepeta cataria</i> (catnip)	leaves (dried, cut)	Frontier / SmartyKat
<i>Valeriana officinalis</i> (valerian)	roots (dried, cut)	Frontier
<i>Camellia sinensis</i> (green tea) ¹	leaves (dried, cut)	Frontier

1594

¹ Used as negative control

1595 **Table 3. An overview of the single compounds used in this study.**

#		Compound	Class ¹	Retention index ²	Source
1	A ³	nepetalactone (<i>cis-trans</i> -nepetalactone)	type II lactone	1383	synthesized
2	A	epinepetalactone (<i>trans-cis</i> -nepetalactone)	type II lactone	1416	synthesized
3	B	dihydronepetalactone	type II lactone	1490	synthesized
4	B	isodihydronepetalactone	type II lactone	1446	synthesized
	B	<i>trans</i> -dihydronepetalactone ⁴	type II lactone	1505	synthesized
	B	<i>trans</i> -isodihydronepetalactone ⁴	type II lactone	1470	synthesized
5	C	neonepetalactone	type II lactone	1517	synthesized
6	C	isoneonepetalactone	type II lactone	1511	synthesized
7	D	iridomyrmecin	type I lactone	1466	synthesized
8	D	isoiridomyrmecin	type I lactone	1478	synthesized
9		actinidine	pyridine	1348	synthesized
10		dihydroactinidiolide	furanone	1562	AK Scientific
11		indole			AK Scientific
12		menthol			GreenHealth
13		methyl salicylate ⁵			TCI Chemicals

1596 ¹ The difference between type I and II lactones is the position of the carbonyl group (Nangia, Prasuna and

1597 Bheema Rao 1997).

1598 ² Linear retention index relative to n-alkanes on a DB-5ms column

1599 ³ The same letters in the second column of this table indicates these compounds are diastereoisomers:

1600 stereoisomers with one or more differing stereocenters resulting in different molecules that are not mirror
1601 images and not superimposable.

1602 ⁴ These compounds were only prepared in small amounts and used as standards in the GC-MS analysis,
1603 but were not used in bioassays with cats.

1604 ⁵ Liquid at room temperature

1605 **Table 4: Ethogram describing body positions and behaviors seen in domestic cats in response to**
1606 **cat-attracting plants or their volatile compounds.**

Body position	Description
standing	The cat is in an upright position with all paws on the ground and the legs extended.
sitting	The cat is sitting in a crouched position: the body is close to the ground, all legs are bent, and the belly is touching or raised slightly off of the ground; crouched down to get a closer look at the object, not to be mistaken with crouching because of fear.
lying on side	The cat lies on her or his left or right side.
lying on back	The cat lies on her or his back.
Behavior	Description
biting ¹	The cat bites the object or has the object in her or his mouth. Sometimes combined with pulling or shaking her or his head.
head rubbing ¹	The cat rubs with her chin, cheek or forehead against the object.
head shaking ¹	The cat shakes her or his head without an object in her or his mouth. Sometimes combined with shaking the rest of the body.
holding ¹	The cat holds an object with one or two paws.
licking	The cat passes her or his tongue over the object.
raking ¹	The cat makes kicking movements with one or both hind legs against the object. Also known as bunny kicking. Typically seen when the cat holds the object with her or his paws or in her or his mouth.
rippling of back ¹	Rippling or rolling motion of the cat's skin in the dorsal lumbosacral region as the underlying cutaneous trunci / panniculus carnosus muscles rhythmically contract and relax. Not to be confused with feline hyperesthesia syndrome.
rolling on side ¹	The cat rolls on her or his side or back, from a sternal or lateral body position, respectively.
twitching of back ¹	Short (fraction of a second), quick contractions of the cutaneous trunci / panniculus carnosus muscles. Distinct (shorter) from rippling of the back, but possibly related.

1607 ¹ See **Supplementary File 1** for a video with examples of these behaviors.

1608 **Table 5. An overview of the fragrances used in this study.**

Company	Fragrance	Type or concentration	Place of purchase
Calvin Klein	Obsession for Men	eau de toilette	(1) Nordstrom, The Shops at La Cantera, San Antonio, Texas, USA. (2) The Fragrance Decant Boutique (decantboutique.com), Little Elm, Texas, USA. (3) USA Fragrance, Ingram Park Mall, San Antonio, Texas, USA
Nina Ricci	L'Air Du Temps	eau de parfum	Amazon
Guy Laroche	Drakkar Noir	eau de toilette	iDimino
Paco Rabanne	Paco Rabanne Pour Homme	eau de toilette	Natural Nutrient
SP Parfums	Civette Intense	eau de parfum	Indie Scents
Matieres Premieres Essentielles	Civet Absolute (synthetic)	1% in absolute ethanol	Perfumer's Apprentice
Firmenich	Civettone	0.1 and 1% civetone in absolute ethanol	Firmenich

1609

1610 **Table 6. An overview of the methodology used for testing the fragrances.**

Fragrance	Day ¹	n	Amount ²	Hours between application and availability
Ethanol (control)	1	2	2 sprays	0
Obsession for Men (source 1)	1	2	2 sprays	0
Drakkar Noir	2	1	2 sprays	0
Obsession for Men (source 2)	2	1	2 sprays	0
L'Air Du Temps	2	1	2 sprays	0
Paco Rabanne Pour Homme	2	1	2 sprays	0
Drakkar Noir	3	2	2 sprays	0
Civette Intense	3	1	2 sprays	0
Obsession for Men (source 1)	4	1	10 sprays	10
Drakkar Noir	4	1	10 sprays	10
L'Air Du Temps	4	1	10 sprays	10
Paco Rabanne Pour Homme	4	1	10 sprays	10
Obsession for Men (source 3)	5	2	2 sprays	0
Civetone ³ (0.1%)	5	2	2 sprays	0
Obsession for Men (source 3)	6	1	2 sprays	0
Obsession for Men (source 3)	6	1	10 sprays	10
Civetone ³ (1%)	7	1	2 sprays	0
Civetone (1%)	7	1	10 sprays	10
Civet Absolute (synthetic) (1%)	8	1	2 sprays	0
Civet Absolute (synthetic) (1%)	8	1	10 sprays	10

1611 ¹ The fragrances were tested on 8 different days. The same numbers in this column means these

1612 fragrances were tested on the same day.

1613 ² Although there is some variation between different atomizers, each spray is roughly equivalent to 100
1614 μ L.

1615 ³ Natural civet is believed to contain about 1% civetone (Endallew and Dagne 2020).

1616 n, number of fabrics available to the cats

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1618

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