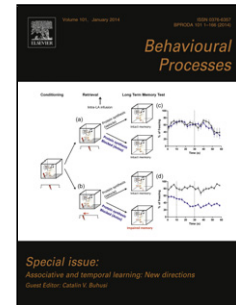


Accepted Manuscript

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PII: S0376-6357(17)30176-6
DOI: <http://dx.doi.org/doi:10.1016/j.beproc.2017.06.008>
Reference: BEPROC 3474

To appear in: *Behavioural Processes*

Received date: 13-4-2017
Revised date: 26-6-2017
Accepted date: 26-6-2017

Please cite this article as: Espín-Iturbe, Luz Teresa, López Yañez, Bernardo A., García, Apolo Carrasco, Canseco-Sedano, Rodolfo, Vázquez-Hernández, Maribel, Coria-Avila, Genaro A., Active and passive responses to catnip (*Nepeta cataria*) are affected by age, sex and early gonadectomy in male and female cats. *Behavioural Processes* <http://dx.doi.org/10.1016/j.beproc.2017.06.008>

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Active and Passive Responses To Catnip (*Nepeta cataria*) Are Affected by Age, Sex and Early Gonadectomy In Male And Female Cats

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Research Highlights

- Only two thirds of adult cats are believed to respond to catnip (*Nepeta cataria*)
- Responsiveness is mainly based on “active” behaviors, such as rolling over
- Herein we assessed active and passive responses in cats of different age, sex, and gonadal status
- Few cats responded actively, but almost 100% did it passively (sphinx-like posture)
- We discuss brain maturation as the cause to catnip response

Abstract

Catnip (Nepeta cataria) is a popular plant among cat owners because in about 60% of felids elicits active behaviors such as rolling over, grooming, motor activity and vocalizations. Herein, we assessed the display of active but also passive responses, such as time in sphinx-like position, and consequently hypothesized that 100% of cats respond to catnip. Accordingly, sixty domestic cats of different age (infant, juvenile, adults), sex (males, females) and gonadal status (early gonadectomized, gonadally intact) were placed in a cylindrical chamber (1.20 X 1.40 m) during 5 min and then exposed to 500 mg of dehydrated catnip for another 5 min. Behaviors were videorecorded and scored. Results indicated that about 20% of the cats (adults and juvenile only) displayed active behaviors (i.e. rolling over), whereas 80% displayed passive responses at any age (sphinx-like position, decreased frequency in vocalizations, and decreased motor activity). These results suggest that all cats respond to catnip but they express it actively, passively or with a combination of both types of responses, which mainly depends on age and sex, and early gonadectomy to a much less extent. We discuss the possible implications of brain maturation on this dichotomy and speculate on the role of opioidergic system on the catnip responses.

Keywords: *Nepeta cataria*; catnip; domestic cat; gonadectomy; age; opioids

1. Introduction

Catnip (*Nepeta cataria*) is a popular plant among cat owners because it elicits a predictable “playful” behavior in most felids. Only a small portion of the plant (about 0.001-0.3%) contains the oil nepetalactone, which is reported to produce the behavioral effects (McElvain, Bright, and Johnson 1941; Bol et al. 2017). Studies indicate that 2/3 of cats exposed to catnip may display behaviors such as rolling over, chin and cheek rubbing, head shaking, pawing, floor scratching, persistent sniffing, licking and chewing of the catnip source (Todd 1962, 1963; Bol et al. 2017). The effects start immediately after exposure and last only for about 5-15 min, followed by a refractory period of non-responsiveness during several minutes (Todd 1963). It has been argued that such responsiveness does not depend on sex or gonadal status (gonadally intact vs. gonadectomized), but only on age, although those observations were obtained from cats gonadectomized in adulthood, not at early age (<3 months) (Todd 1963; Grognet 1990). A similar response is observable in some large felids, being lions and jaguars extremely sensitive to the behavioral effects (up to 60 min) compared to tigers, cougars, bobcats and oncilla cats that express little or no response (Hill et al. 1976; Resende Lde et al. 2011).

Several years ago it was argued that responders (65% of domestic cats) may have a predisposition to react to catnip, presumably because of an inherited dominant autosomal gene (Todd 1962). Such classification was focused on the display of “rolling over” behavior, and the coincidence of such response to the one expressed by their ancestors. Some years later Hill and colleagues observed large felids and suggested that the expression of rolling over behavior is an insufficient criterion for determining responsiveness since it ignores other frequently elicited passive behaviors (i.e. lying/sitting) (Hill et al. 1976). Accordingly, former studies considered that catnip

response occurred when cats behaved actively via rolling over, chin and cheek rubbing, head shaking, pawing, floor scratching, persistent sniffing, and licking. However, we believe that many cats referred to as active non-responders may indeed be passive responders if they display explicit passive behaviors (i.e. more time in sphinx-like posture) starting soon after exposure to catnip. In addition, passive catnip responses may be observed when regular baseline activity is decreased (e.g. less motor activity, less grooming, less vocalizations). Thus, in this study we hypothesized that all cats can express a response, which may be either active, passive or mixed. In addition, we hypothesized that regardless of the type, the response would be affected by age, sex or early gonadectomy.

2. Methods

2.1 Subjects

A total of sixty domestic cats (*Felis silvestris catus*), pending for adoption in a local animal shelter at Veracruz city, Veracruz Mexico, were used for this study. All the procedures were approved by an admission committee of the Graduate Program in Neuroethology, Universidad Veracruzana and were carefully controlled to minimize distress according to the official Mexican norm NOM-062-ZOO-1999. Cats were classified by age, based on the shelter records and were subsequently organized into three groups: Infant (age <3 months old N=20), Juvenile (age 3-6 months old N=20), and Adults (age >6 months old N=20). Groups consisted of equal number of males and females, and equal number of gonadally intact or early gonadectomised animals.

2.2 Gonadectomy

A veterinarian in the facilities of the shelter performed gonadectomy. Cats (6 weeks old) were anesthetized with a mixture of ketamine hydrochloride (10 mg/kg) and xylazine hydrochloride (3 mg/kg), injected via intramuscular (i.m.). Anesthetized females were ovary-hysterectomized via an abdominal, middle line incision. Males were castrated via a scrotal incision. Post-surgical treatment included three days of tolfenamic acid (4 mg/kg i.m.) for analgesia, and five days of enrofloxacin (5 mg/kg i.m.) every 24 h to prevent post-surgical bacterial infections. All animals were given at least two weeks of post-surgical recovery before their behavior was assessed following exposure to catnip.

2.3 Apparatus

Behavioral observation occurred in a cylindrical chamber (1.20 mØ X 1.40 m height), which prevented cats from watching their surrounding and escaping. The chamber was made out of strong wire and its walls were covered by white tarp. The cylindrical chamber was placed in one isolated room at the shelter, protected from direct sunlight and excessive noise. The chamber floor was divided (using masking tape) in quadrants of 20x20 cm. So, changes of quadrants functioned to calculate motor activity. All the cats were habituated to this chamber 10 min every day, during three trials. On the morning of the fourth day each animal was brought into the room, placed into the chamber and left undisturbed during 5 minutes to video record baseline behaviors from above the chamber. Then each cat was exposed to 500 mg of commercial dehydrated leaves of catnip and their behavior was video-recorded for 5 more minutes and scored using a computerized software (Behavioral Observation Program (Cabilio 1998)).

2.4 Catnip

Commercial catnip was used for this study (Whisker city®, Pacific Coast Distributing, Phoenix, AZ). Prior to the start of the experiment few random samples from a homogenized bag of catnip were tested by analytical chemistry for its content of nepetalactone in a certified laboratory (SARA, Universidad Veracruzana). A catnip sample (0.5 g) was extracted with hexane and chloroform. Extracts were analyzed by gas chromatography coupled with a mass selective detector (GC-MS, Agilent Technologies Model 6890N Mass spectrometer model 5975 inert XL). The GC system was equipped with a Varian carbowax column (30 m, 0.25 mm internal diameter, 0.25 µm film). Conditions were as follows: inlet pressure: 67 kPa, He-flow: 1 ml/min, injector: 250 °C, transfer line 250 °C, electron ionization energy 70 eV. The GC oven temperature was kept at 80 °C for 5 min, followed by increases in temperature 30°C/min until 250 °C. Identification of compounds was performed by comparison of their mass spectra and retention indices. The chromatographic results obtained from hexanic and chloroformic extracts showed two fractions with 11.83 and 12.2 min of retention, respectively. The two fractions corresponded to nepetalactone, considering its molecular weight of 166 and the fragmentation patterns described in the literature (Safaei-Ghomi, Djafari-Bidgoli, and Batooli 2009). Thus, we confirmed that our samples were obtained from a batch that contained catnip.

2.5 Behavior and data analyses

Catnip response was expected to last few minutes only (Todd 1963). Thus, behaviors were analyzed exclusively 5 min before exposure to catnip (A) and 5 min following exposure (B). All the cats received 500 mg of the dehydrated commercial catnip bag (Whisker city®, Pacific Coast Distributing, Phoenix, AZ). During behavioral testing each sample was placed on a piece of bond paper and then introduced into the

chamber and left on the floor. The chamber's floor was cleaned with water and paper towels after every cat. Behaviors were chosen on the idea that cats might be active or passive responders. Thus, we measure the frequency (f) of explicit active behaviors such as rolling over, self-grooming, vocalizations, and motor activity (as observed with change of quadrants in the chamber's floor). In addition, we measured what we considered an explicit passive response like the time spent (seconds) in sphinx-like position (**Figure 2C**). We considered that a cat that adopts a sphinx-like posture is responding with certain motor and motivational control. Furthermore, passive-like effects were also assumed when the frequency of some active behaviors (self-grooming, vocalizations, and motor activity) was decreased upon exposure to catnip. Thus, the difference between [B-A] was then processed with a three-way (2 x 2 x 3) analysis of variance (ANOVA) to assess the general effects sex (male vs. female), gonadal status (intact vs. early gonadectomised) and age (<3 months, 3-6 months, >6 months) on the catnip response. For all significant main effects or interactions detected, an LSD *post hoc* test was conducted to assess differences between individual means. The alpha level was set at $p < 0.05$. All the statistics were processed in Prism 7 for Mac OS X.

3 Results

As expected, all cats responded to catnip with either active or passive behaviors. The ANOVA showed an increase in rolling over $F(1, 58) = 13.99, p = 0.00$; sphinx-like position $F(1, 58) = 73.99, p = 0.00$; and grooming $F(1, 58) = 53.12, p = 0.00$, and a decrease in vocalizations $F(1, 58) = 30.87, p = 0.00$; and motor activity $F(1, 58) = 19.07, p = 0.00$ (see **table 1**). These behaviors, however, were mainly affected by the cats age, and sex (**table 2**). For instance, rolling over behavior was mainly affected by age $F(2, 48) = 6.027, p = 0.0046$. The *post hoc* analysis indicated that adult cats (>6

month old) displayed more rolling over than juvenile cats (3-6 month old), but none of the infants displayed this behavior (<3 month old) (**Figure 1A-B**). Infants, however, decreased their motor activity, and displayed sphinx-like position upon exposure to catnip, although for sphinx-like position there was also a main effect of age $F(2, 48) = 6.457, p=0.0033$ and an interaction between age and sex $F(2, 48) = 4.564, p=0.0153$. The *post hoc* analysis indicated that sphinx-like position was more likely observed in adults than in juvenile and infants (**Figure 2A**), but juvenile males (3-6 months) were more likely to express it than juvenile females (**Figure 2B-C**). For grooming there was a main effect of age $F(2, 48) = 9.409, p=0.0004$ and sex $F(1, 48) = 5.947, p=0.0185$. Following exposure to catnip, adult cats expressed more grooming than juvenile, and these more than infants (**Figure 3A**). In general, females responded with more grooming than males after exposure to catnip (**Figure 3B**). There was a main effect of sex for the reduction in vocalization frequency $F(1, 48) = 4.587, p=0.0373$, indicating that females vocalized less than males after exposure catnip. In addition, there was an interaction between age and sex $F(2, 48) = 3.479, p=0.0388$, indicating that infant females vocalized less than males, but the difference was not observed in adults. For motor activity there was a main effect of sex $F(1, 48) = 5.69, p=0.0211$, and early gonadectomy $F(1, 48) = 5.844, p=0.0195$, thus males moved less than females, and early-gonadectomized cats moved less than gonadally-intact after catnip.

Tables 01 and 02 about here

Figures 1-3 about here

Discussion

The present study indicates that exposure to catnip did induce both active and passive responses in domestic cats from a shelter. Active effects were observed through an immediate increase in the frequency of behaviors such as rolling over and grooming; whereas passive effects were observed with more time in sphinx-like posture, less vocalizations and less motor activity as compared to baseline levels. To some extent, all these responses were affected by either age, sex or gonadal status.

Our results support the former idea that not all cats display the active rolling over behavior (Todd 1963; Grognet 1990), but we also provide evidence indicating that this behavior is insufficient to assess responsiveness. Rolling over depended strongly on age, but not on sex or gonadal status. Adult cats (45%) and few juvenile ones (25%) were good rolling-over responders, but not infants (0%) (**Figure 1A**). Nevertheless, other passive responses were certainly observed in most of the cats (>95%), such as a reduction in both motor activity and vocalization frequency, or an increased of time in sphinx-like posture. These behaviors may indicate that almost 100% of individuals reacted to catnip, but many of them did it passively. Perhaps, the transition from expressing only passive to active responses increases with age, and such capacity is then selectively tuned up by sex or gonadal status. For instance, we observed that sex was important to predict the catnip response. As compared to females, males reacted with more time in sphinx-like position, less grooming, less vocalizations and less motor inactivity. Likewise, we observed that early gonadectomy had an effect on the response. Cats that underwent gonadectomy before 3 months of age showed more inactivity than gonadally intact animals following exposure to catnip. Thus, age appears to be the main factor for responsiveness, followed by sex and then to a much less extent early gonadectomy. Former studies argued that male and female cats responded equally to catnip, regardless of their gonadal status. However, such observations were based on the

rolling over response, and also in individuals that were gonadectomized in adulthood only (6-9 months of age) (Grognet 1990). Further research using a larger number of animals is needed to fully understand the effects of early gonadectomy (<3 month old) vs. adult gonadectomy (>6 months old) on the catnip response. It is important to point out that during this study cats were not presented to an additional control odorant to distinguish between responses specific to catnip and those more associated with olfactory inspection. This may be particularly important for the discussion of behaviors that decreased (i.e vocalizations and motor activity) probably as a result of being distracted by the odor. However, we do not consider this a limitation of the study because other observed behaviors would not support a “novel odorant effect”. For instance, rolling over, grooming or sphinx-like posture.

Age as a main factor

Cats expressed more active responses in adulthood (**Figures 1A, 3A**). At first, it may suggest that hormones caused such effect. However, the frequency of rolling over and grooming was not modified by the presence of a functional gonad (as observed in intact animals). Only motor inactivity (reduction of change of quadrants evoked by catnip) was directly enhanced by early gonadectomy. Thus, differences in the levels of gonadal hormones are not sufficient to explain the occurrence of all behaviors in adulthood. Age, however, correlates positively with brain maturation, which may refer to consecutive changes of brain weight, increment in the number of brain cells (glia), expansion of dendritic arborization, interconnectivity with other neurons and changes in receptors density. Those brain changes may explain how at juvenile age the cat starts to become a fully independent carnivore, with a high degree of sensory and motor specialization, as well as cognitive abilities associated with orientation in a complex

environment that requires prey-catching. Thus, one can suppose that its brain is becoming mature by this age, which may explain active responses after catnip. Before three months of age, the brain is not completely mature and cats may express passive responses only. The idea of brain maturation as a possible cause of responsiveness may be also supported by other passive behaviors that were observed in adults but less frequently in juvenile and infants (e.g. more time in sphinx-like position, and decrease of vocalizations) (**Figure 2A**). We speculate that maturation of the opioidergic system may account for the observed differences in behavior between infant, juvenile and adult cats after catnip.

Opioid/Reward circuitry activation following catnip

Former studies argued that adult cats may experience a copulatory-like after reaction following exposure to catnip due to the apparent similarity with the rolling over, chin rubbing and sphinx-like position that female cats display after mating (Palen and Goddard 1966). Such idea is in accordance with the age effect because only adult cats are reproductively mature, but is not in accordance with sex or gonadal status. In other words, sexual behavior requires hormone priming, whereas catnip response does not, and both sexes respond to catnip, not only females. Interestingly, one study showed that exposure to catnip can change the pattern of discharge of cells in the ventromedial nucleus of the hypothalamus (VMH) (Campbell et al. 1969). The VMH is a brain area important for mate recognition and sexual behavior in ferrets and rodents (Pfaff et al. 2011; Robarts and Baum 2007), and it is activated during sex (Georgescu et al. 2009). Thus, catnip might activate VMH neurons even in absence of gonadal hormones and therefore mimic its activation during mating.

The VMH expresses different types of opioid receptors which have been shown to regulate female receptivity (Acosta-Martinez and Etgen 2002, 2002). Opioids are part of the neurochemical substrate that underlies different types of reward, like sexual (Herz 1998; Garcia-Horsman, Agmo, and Paredes 2008), and perhaps catnip-induced reward as well. Evidence indicates that essential oils extracted from plants of the *Nepeta spp.* family may function as opioid-like analgesic/sedatives because their effects are blocked by treatment with the opioid antagonist naloxone (Aydin et al. 1998). The parallel between catnip and opioids may be also observed in behavioral responses after exposure to these two kind of substances. For example, one study showed that low doses of morphine (0.5-3 mg/kg) produced three types of behavioral responses in cats, which were categorized in three stages. The first stage was referred to as *autonomic* and occurred 0-15 min postdrug. Cats in the *autonomic* stage displayed vocalizations, salivation, licking, swallowing, retching and vomiting. The second stage was referred to as *quiet* and occurred 15-60 min postdrug. Cats displayed sitting, fixed gaze, mydriasis, and erected ears. Finally, the third stage was referred to as *head movement stage*, and occurred 30-60 min postdrug. In this stage cats were fully aroused but mostly sitting; showing discrete, complex head movements with pouncing/avoidance paw movements, and with irregular bouts of rocking, pivoting, and backing (Villablanca et al. 1984). Interestingly, a study also found that age affected the behavioral responsiveness. For example, kittens (<2 months old) did not display the *autonomic* stage, which involves more active behaviors. Only adults displayed the complete responses of the three stages (Burgess and Villablanca 2007). In addition, evidence indicates that endogenous opioids are not completely functional in young cats <2 months old (Thor et al. 1990). As observed in the present study catnip did not affect certain behaviors in infants either.

Thus, catnip response may depend on the development of the opioidergic system in the brain, but further studies are required to confirm this hypothesis.

Other chemicals with potential effect

Bol and colleagues (2017) argued that cis-trans nepetalactone might be the dominant and possibly the only compound to which cats respond, due to its highest concentration in dry leaves (found in their study in a concentration of 1010 $\mu\text{g}/\text{gram}$). However, they also reported that catnip contains other chemicals with potential effects on behavior, such as isodihydronepetalactone (55 $\mu\text{g}/\text{gram}$), trans-cis nepetalactone (32 $\mu\text{g}/\text{gram}$), and actinidine (11 $\mu\text{g}/\text{gram}$) (Bol et al. 2017). The high concentrations of actinidine in plants such as silver vine, tatarian honeysuckle and valerian root is believed to explain cats' responsiveness to those plants. Nepetalactones are essentially bicyclic monoterpenes, which possess gamma amino butyric acid (GABA) modulatory effect (Tambe et al. 2016), whereas actinidine is a monoterpene alkaloid that can inhibit monoamino oxidases (MAO) A and B (Dos Santos Passos et al. 2013) and might therefore result in increased levels of brain monoamines like dopamine and noradrenaline. Accordingly, GABA activity results in inhibition of neural activity, and may be the cause of passive responses, whereas enhanced levels of monoamines can result in increased neural activity, and may be the cause of active responses.

Catnip responsiveness as predictor of temperament

Assessment of the catnip responsiveness might be a useful tool as well, perhaps to predict temperament associated with brain chemistry. For example, a recent study argued that some cats can be categorized in either: 1) Cat social, 2) Active, 3) Human nonsocial, 4) Human aggressive, or 5) Intense (Ha and Ha 2017), which can be

modulated by variables such as outdoor usage, feeding style (*ad libitum* vs. meal fed), cohabiting with other cats, sex, time owning the cat, and previous history as a stray. Because temperament results from brain chemistry we hypothesize that a cat's temperament might also correlate somehow with the catnip response. For example, it may be the case that cats categorized as active, human nonsocial, aggressive, or intense may respond more actively to catnip, whereas those cats categorized as cat social, may respond more passively. Further research is needed to confirm this hypothesis. If this were true, catnip response could be used as a quick method for temperament assessment or perhaps to gather an idea of brain maturation.

6. Conclusion

The response to catnip can be classified into active or passive, depending on the display of behaviors. The former was mainly observed as increment in the frequency of rolling over and grooming, whereas passive responses were mainly observed via a decrease of motor activity, less frequency of vocalizations, and more time spent in sphinx-like posture. Active responses were observed in adults, but not in infants. Such repertoire correlates with some behaviors observed in cats that receive systemic opioids, suggesting that active behaviors may be a consequence of a mature opioidergic system. However, catnip can potentially mediate some effects on GABA and brain amines as well, regulating passive and active responses, respectively.

Acknowledgements

We would like to thank to “Albergue del Centro de Salud Animal” and “Parque Ecológico, Miguel Ángel de Quevedo” for their support to this study.

Conflicts of interest

The authors declare no conflict of interest

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Figure legends

Figure 1. Catnip-induced rolling over is mainly affected by age (A), but not by sex (B), or gonadal status (not shown in figure). Adults (>6 months old) display more rolling over than in juvenile cats (3-6 months old). Infants (<3 months) do not display it following exposure to catnip. The mean difference is obtained from the frequency observed after exposure to catnip minus the frequency observed before exposure. Different letters indicate significant differences.

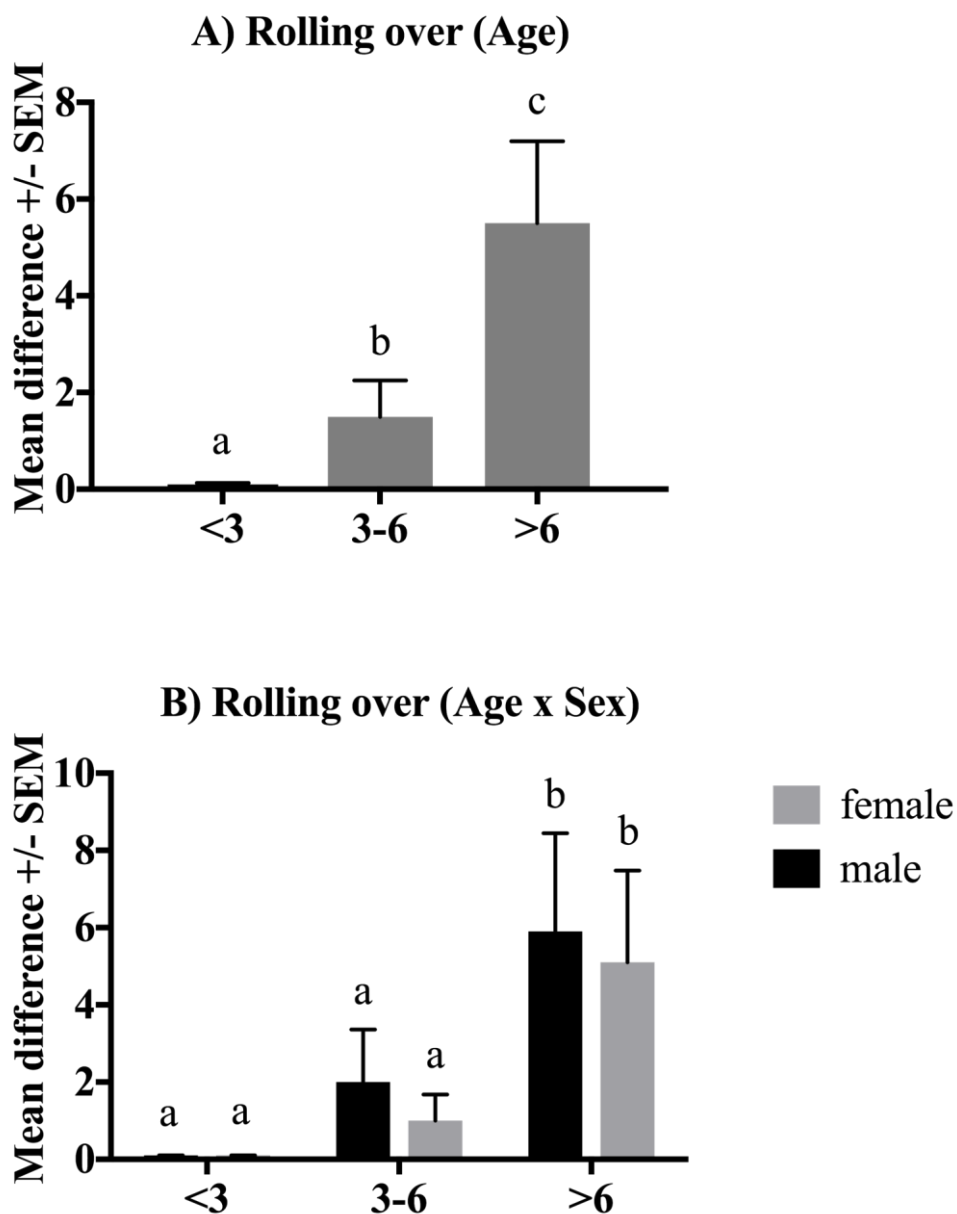


Figure 2. Sphinx-like position (passive response) is observed after exposure to catnip. A) It is mainly affected by age. B) Juvenile males start to express this passive response earlier than females between 3-6 months old. Different letters indicate significant differences.

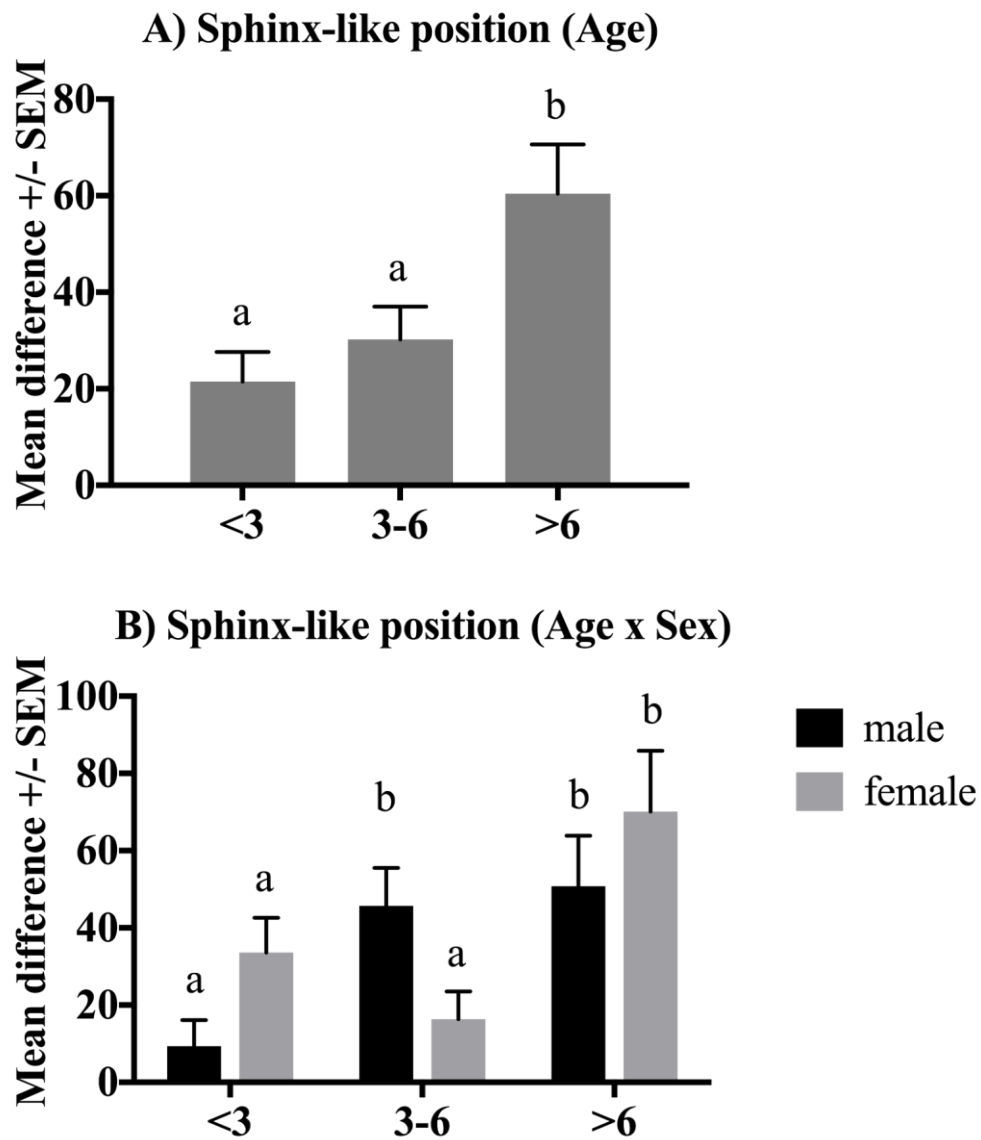
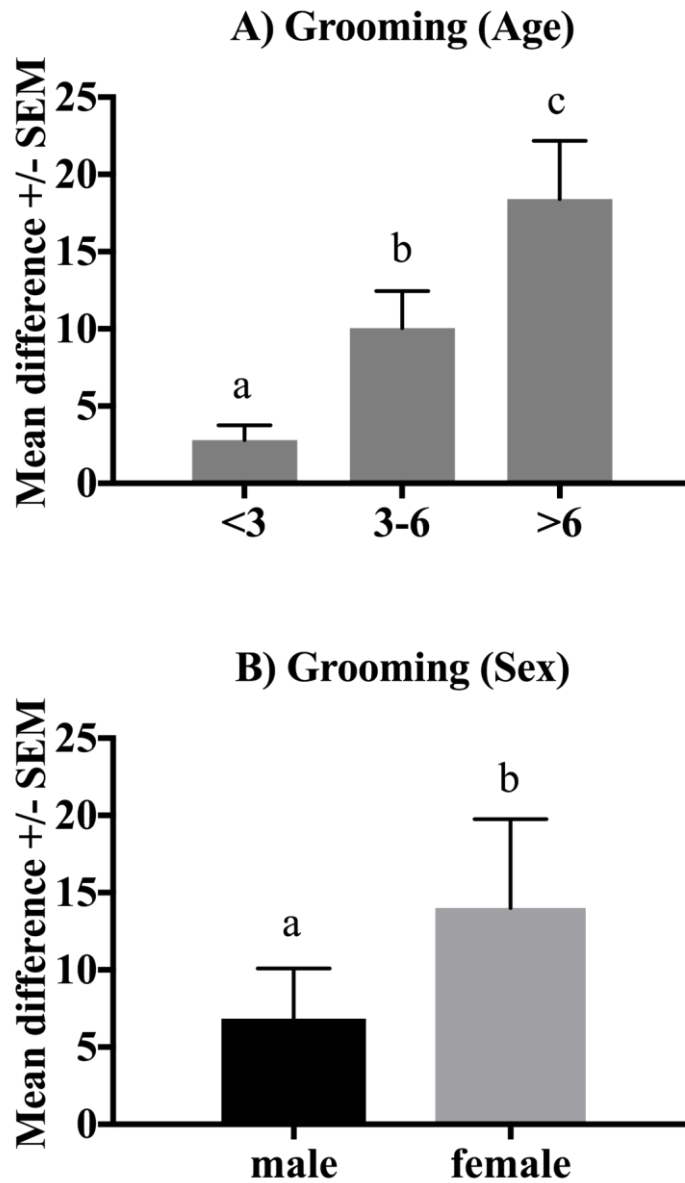




Figure 3. Catnip-induced self-grooming. A) It is mainly affected by age, so adults express this behavior more than juvenile, and these more than infants. B) Females respond with more grooming than males after exposure to catnip. Different letters indicate significant differences.



General effects of catnip 1

Behavior	Before catnip	After catnip	General Effect	Influenced by		
				Age	Sex	GS
Rolling-over (f)	0.00 ± 0.00	2.40 ± 0.68*	↑	yes	no	no
Sphinx-like position (s)	0.00 ± 0.00	38.77 ± 5.09*	↑	yes	yes	no
Grooming (f)	0.60 ± 0.28	11.02 ± 1.67*	↑	yes	yes	no
Vocalizations (f)	28.45 ± 2.81	16.37 ± 1.75*	↓	yes	yes	no
Motor activity (f)	23.82 ± 1.27	16.60 ± 1.11*	↓	no	yes	yes

Table 1. Mean ± SEM of behaviors assessed in a cylindrical chamber during 5 minutes before vs. after exposure to catnip (500 mg dehydrated leaves). f= frequency, s= seconds. * indicates $p < 0.05$ using a three-way (Age, Sex, GS) ANOVA. N=60. ↑= increase, ↓= decrease, GS= gonadal status (intact vs. early gonadectomy).

Effects of Age, Sex and Gonadectomy 1

Behavior	Intact						Early		
	Female			Male			Female		
Sex									
Age	<3	3-6	>6	<3	3-6	>6	<3	3-6	>6
Rolling over	0 ± 0	4 ± 2	2.4 ± 1.6	0 ± 0	2 ± 1	5 ± 2	0 ± 0	0.8 ± 0.8	7 ± 3
Sphinx-like position	48.6 ± 9	8 ± 8	65.6 ± 29	18.8 ± 12	39 ± 16	56.4 ± 17	18.6 ± 12	24.8 ± 11	74 ± 11
Vocalization	30 ± 4	17.8 ± 2	8.8 ± 2	37 ± 6	14.8 ± 2	11.2 ± 2	35.8 ± 5	16 ± 2	6 ± 3
Motor activity	9.4 ± 2	18.2 ± 4	20 ± 0.6	18.2 ± 3	22.4 ± 3	13 ± 2	10.4 ± 3	17 ± 1	19.6 ± 3
Grooming	6 ± 3	20 ± 3	19.6 ± 3	1.4 ± 0.8	7.6 ± 3	11.8 ± 7	3.2 ± 1	7 ± 3	30.4 ± 3

Table 2. Mean difference ± SEM of behaviors induced by exposure to catnip minus before exposure (column n= 5). A three-way ANOVA was used to detect significant differences of age, sex, and gonadal status. See the results section.