Dogs, but not wolves, lose their sensitivity towards novelty with age

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Abstract

Selection on behavioural traits holds a prominent role in the domestication of animals. Specifically, a reduction of the fear response is considered a key component, with domesticated animals expressing lower levels of fear towards novelty than their wild counterparts. Previous work has suggested that this is caused by a delay in the onset of fearful behaviour during early ontogeny in domesticated canids. However, it remains unclear how the developmental timing of initial fear expression affects fearfulness later in development. Here we present the first extended examination of the development of fear behaviour in wolves and dogs, using repeated novel object tests between six and 26 weeks of age. Contrary to expectations, fear of novelty did not change in wolves with age, but dogs expressed decreased latency to approach a novel object with age, resulting in a species difference at the end of the measured period. Our results thereby suggest that differences in fear of novelty between wolves and dogs are not caused by a domestication driven shift in the first onset of fear response. Instead we suggest that differences in fear expression between wolves and dogs are caused by a loss of sensitivity towards novelty with age in dogs.

Key words: *Fear, domestication, sensitive period, ontogeny, novel object test, Canis*

Introduction

Abundant evidence demonstrates how domesticated plants and animals express dramatically altered phenotypes compared to their wild counterparts (1). For animals, it is now clear that selection on behavioural traits alone had a prominent role in domestication (2-4). Specifically, it has been demonstrated how selection upon decreased fearfulness and aggression can lead to the myriad of morphological and physiological alterations observed in domesticated animals (2,5). In wild populations, fear is a key behaviour, as a timely and proper response to novelty, e.g. flight response versus exploration, can have direct and large fitness consequences (6,7). Appropriate fear responses are formed and modified throughout ontogeny, during which juvenile animals gradually combine individual experience and social information, thereby developing the ability to discriminate between threatening and neutral stimuli (7-9). Domesticated animals express reduced reactivity towards novel stimuli (10) and it is thus likely that altered selection pressures caused by domestication have modified the ontogeny of fear related behaviours.

Ontogeny has been modified in several ways during domestication. Compared to ancestral species, domesticated animals express altered developmental rates, a phenomenon known as heterochrony (11-13), which have resulted in accelerated and/or delayed onsets of various ontogenetic stages, such as earlier sexual maturation and the retention of juvenile traits into adulthood (12,14-16). Heterochrony has been suggested to affect behavioural ontogeny in domesticated animals by prolonging the sensitive period (2,17-19), an important period during behavioural development in which the juvenile animal is particularly sensitive to imprint on and form social bonds with conspecifics (8,20-22). During the sensitive period juvenile animals show increased exploratory behaviour, as they readily approach novel stimuli and thereby learn about and socialize with their environment

(23). Importantly, in the context of the ontogeny of fear, the end of the sensitive period manifests by progressive fear-related avoidance behaviour expressed as increased fear and decreased exploration of novelty (2,21). A shift in the sensitive period caused by domestication has been demonstrated in a long-term selection study, in which juvenile foxes (*Vulpes vulpes*) from domesticated strains expressed a delayed onset of fearful response, at 60-65 days of age, compared to the onset in non-domesticated fox kits, at 40-45 days of age (2,5). While these findings suggest that the basis for the quantifiable difference in fear between domesticated and non-domesticated animals might arise already during early ontogeny, no study has investigated the continued ontogenetic trajectory of fear behaviour after the initial onset of the fearful response.

The domestic dog (*Canis familiaris*) is an excellent study species when addressing questions about how domestication has affected the ontogeny of behaviour. Domestication of the dog from the grey wolf (*Canis lupus*) occurred at least 15,000 years ago (1), making the dog the first domesticated species and with the ancestral species extant, the opportunities for comparisons are ideal (24). Studies of behavioural ontogeny in dogs have focused on the sensitive period, and the fear of novelty is reported to manifest and continually increase in the dog puppy from eight weeks of age onward (8,21,25,26). In wolves, consensus on when fear behaviour is established is lacking, with the onset of fearful response reported to occur as varied as four to eight weeks of age across studies (25,27-31). However, the majority of these wolf studies were conducted over a short period of time and/or focused on isolated individuals or single litters, limiting our ability to generalize from these findings. Additionally, a recent study found that juvenile wolves explored novel objects more than dogs at both six and eight weeks of age (32), thereby suggesting that that wolves might not express fear towards novelty at an earlier age than dogs.

Thus, while adult wolves (33) and wolf-dog hybrids (34) are more fearful of novelty than dogs, when this difference is established during ontogeny is not well resolved. Therefore, extended studies of the continued development of fear after the onset of fearful response is needed to further understand domestication driven changes in behavioural ontogeny in canids.

To investigate if behavioural differences in fear towards novelty differ between wolves and dogs during the first six months of life, we here examine their behavioural development by using repeated novel object tests. The novel object test is an established method to quantify fear and exploration of novelty and has been used on numerous species (32,33,35-37) We tested three litters of wolves (N = 13) and two litters of dogs (N = 12), hand-raised under similar conditions at six, 10, 14, 18, 22 and 26 weeks of age, i.e. before sexual maturity, and varied the context by using a new novel object in each of the six tests. Our overall goal was to test the hypothesis that the sensitive period is prolonged in dogs, resulting in a delayed onset of fearful response in dogs compared to wolves. Based on studies reporting delayed onset of fear behaviour in domestic compared to ancestral species (2,38), including dogs and wolves (22,31), we expected wolves to be more fearful compared to dogs already at six weeks or, alternatively, at ten weeks of age. Furthermore, we wished to investigate how different timing of the onset of fearful response affects the continued behavioural development of fear in wolves and dogs. We predicted that domestication has lowered the sensitivity to novelty in dogs (33,39), and dogs therefore would express decreased fear towards the novel object compared to wolves throughout the testing period.

Methods

a) Study animals

During 2014 – 2016 two litters of Alaskan huskies (N = 12) and three litters of European grey wolves (N = 13) were hand-raised and extensively socialized under similar conditions from the age of 10 days at Stockholm University's Zoological Research Station, Tovetorp. This set-up was chosen to minimize environmental bias, including maternal effects, which is well-documented to affect the development of behavioural patterns (40-42). Puppies were raised within litters and socialization involved 24-hour presence of human caregivers for the first two months. From two months of age, caregiver presence was decreased with a few hours a day until three months of age and then further decreased during every other night at four months of age. At six months of age, caregivers spent four to six hours with the puppies a day. All wolf and dog litters were kept separate, but reared under standardized conditions. From the age of 10 days to five weeks, puppies were reared in identical indoor rooms and here after given access to smaller roofed outdoor enclosures. After a week of habituation to the roofed outdoor enclosure, puppies were given access to a larger fenced grass enclosure at six weeks of age. Hereafter the puppies had free access to all three enclosures during the day and access to the indoor room and the roofed enclosure during the night. When the puppies where three months old they were moved to large outdoor enclosures (2,000 square meters), in which they remained for the rest of the study period. We started behavioural observations at 10 days of age and behavioural testing was initiated at 6 weeks of age. Testing procedures and exposure to the new environments were standardized over all three years. As required by Swedish law, all hand-raisers were ethically certified and trained to handle animals. Furthermore, rules were implemented to assure that rearing was standardized across all caregivers. This included that puppies were never disciplined, trained or forced to have contact with their caregivers. From the

136 age of eight weeks, puppies were gradually exposed to strangers through the fence
137 with the support of one or more human caregivers.
138
139 The dog litter from 2014 consisted of five males and one female and the 2015 litter of
140 three males and three females. The two dog litters were unrelated. The wolf litters
141 from 2014, three females and two males, and 2015, two males, were full siblings. The
142 third wolf litter from 2016 consisted of four males and two females and was
143 unrelated to the wolf litters from 2014 and 2015.
144
145 *b) Behavioural sampling*
146 To ensure that the puppies' senses were fully developed by the time of the first test
147 we conducted the first novel object test at six weeks of age (31). Novel object tests
148 were hereafter performed on a monthly basis at 10, 14, 18, 22 and 26 weeks of age.
149 The puppies were tested in a familiar room (4x4 meters) in which a novel object was
150 present, placed opposite of where the puppy would enter the room, approximately
151 four meters away. Puppies were lead into the room by a caregiver, who quickly left
152 the room and closed the door. The duration of a trial was 10 minutes and trials were
153 always monitored by CHW. Some trials (n = 11) were stopped prematurely because
154 the novel object was destroyed. All test were filmed with two mounted GoPro
155 cameras (model 3-4, GoPro Inc.) on opposite sides of the room.
156
157 *c) Novel objects*
158 A different novel object was used at each age in order to avoid habituation. Objects
159 were handled as little as possible and always with freshly washed hands to avoid
160 food smells transferring to the objects and possibly affecting the puppy's behaviour
161 towards the object. Novel objects were chosen based on previous studies with
162 wolves and dogs (Moretti et al. 2015, Marshall-Pescini et al. 2017) and varied on

several scales, such as shape, sound and movement, and included at six weeks: a rolled up mattress, 10 weeks: a wheelbarrow (up-side down), 14 weeks: a mirror mounted to the wall, 18 weeks: a stuffed wolverine toy, 22 weeks: a moving mechanical dog and 24 weeks: a moving sheet (attached to a string).

d) Behavioural scoring

Behavioural scoring was carried out using the software BORIS v. 5.1.3. (43) based on an ethogram (Table 1). Behaviours were logged both as frequencies and durations (i.e. state behaviours). Similar to previous studies (Moretti et al., 2015), latency to approach the novel object was measured as the duration from test start to the time the puppy came within 1 meters distance of the novel object (Table S1). Latency to make contact with the novel object was measured as the duration to make physical contact with the novel object for the first time after the latency to approach (Table S1). Behaviours were scored in a non-overlapping way, with prioritization of behaviours related to the novel object, i.e. if the puppy was looking at the novel object while walking around the test room this was scored as *looking at novel object*.

Table 1. Ethogram. *Behaviours scored during novel object tests. Behaviours were scored in a non-overlapping way, with prioritization of behaviours related to the novel object. Latency times were measured regardless of the behaviour performed.*

Behaviour	Code	Description
Active behaviour	A	Moving around in, or interacting with, the test room with no attention to the novel object
Investigating novel object	I	Sniffing novel object or looking novel object form less than 1 meter
Latency to approach novel object	-	Time delay to approach the novel object with less than 1 meter
Latency make contact with novel object	-	Time delay to physically touch the novel object (sniffing)
Looking at novel object	L	Looking at novel object from a distance of more than 1 meter
Manipulating novel object	M	Pawing, nosing, scratching, biting, carrying, standing on novel object
Passive behaviour	P	Standing, sitting or lying passively with no attention to the novel object or the test room, including by the door

Because there were cases where the total duration of the test was less than 10 minutes, the total test duration was included for use in further analysis (Table S2 and S3). In cases where a puppy did not approach the novel object, the trial duration was used as a measure of latency and in cases where the puppy did not make contact with the novel object this was recorded as a missing value. Inter-rater reliability was calculated using Cohen's kappa and was considered good with a value of 87.4%.

e) Statistical methods

We tested for species effects in each behaviour by fitting linear mixed models, with either latency or the time spent on a behaviour as the dependent variable. The fixed effects of interest were species, age, their interaction and sex. Additionally, for the models of time spent, we controlled for variation in the duration of each trial by including duration as a covariate. To account for the repeated measures of individuals and the non-independence of individuals from the same litter, we included random intercepts for both factors. The full model in lme4 syntax: y ~ species * age + sex + duration + (1|individual) + (1|litter). Models were then reduced by backwards model selection using AIC (cut-off □AIC > 2, Table S4), where the parameters for species, duration and the random effects were always maintained. Both latencies were $\log_{10}$ transformed, and the time spent looking, investigating and manipulating the novel object were log transformed after adding 1, in order to fulfil the assumption of normality in the model residuals. We centred the age variable to aid interpretation of the species effect in case of an interaction. When the interaction was retained in the model, we additionally fitted a model where age was a discrete variable, and used that to perform post-hoc tests for species differences at each age (Table S5). All p-values were obtained using Satterwaithe's

approximation of denominator degrees of freedom. Post-hoc p-values were corrected for multiple comparisons using the Holm method.

All statistical analyses were performed in R (v3.4.3, R Core Team 2016), with mixed effects models fitted using *lme4* v. 1.1-15 (44), Satterwaithe's approximation from *lmerTest* v. 2.0-36 (45) and post-hoc testing using *emmeans* v. 1.1.2 (46).

d) Ethical note

Daily care and testing were performed in accordance with ethical legislation under Swedish Law. This study was approved by the Ethical Committee in Uppsala (C72/14), and our facilities and daily care routines were approved by the Swedish Board of Agriculture (5.2.18-12309/13).

Results

Latency measures

We found that wolves and dogs developed differently in latency to approach the novel object within 1 meter, where dogs expressed a larger reduction in latency with age compared to wolves ($t = 2.35$, $df = 120.046$, $p = 0.02$, Table 2, Figure 1a and 2). Dogs significantly decreased their latency with time, while wolves did not (see table S6 for slopes per species), resulting in dogs expressing significantly lowered latency to approach at 26 weeks compared to wolves ($t = -3.131$, $df = 18.666$, $p = 0.006$, $p_{adjusted} = 0.034$, Table S5). At younger ages we failed to detect significant differences in latency to approach the novel object between dogs and wolves (Table S5). For the latency to make contact with the novel object, we found no differences in wolves and dogs ($t = 1.931$, $df = 2.16$, $p = 0.186$, Table 2, Figure 1b and 2), neither did we find evidence of sex differences in either species.

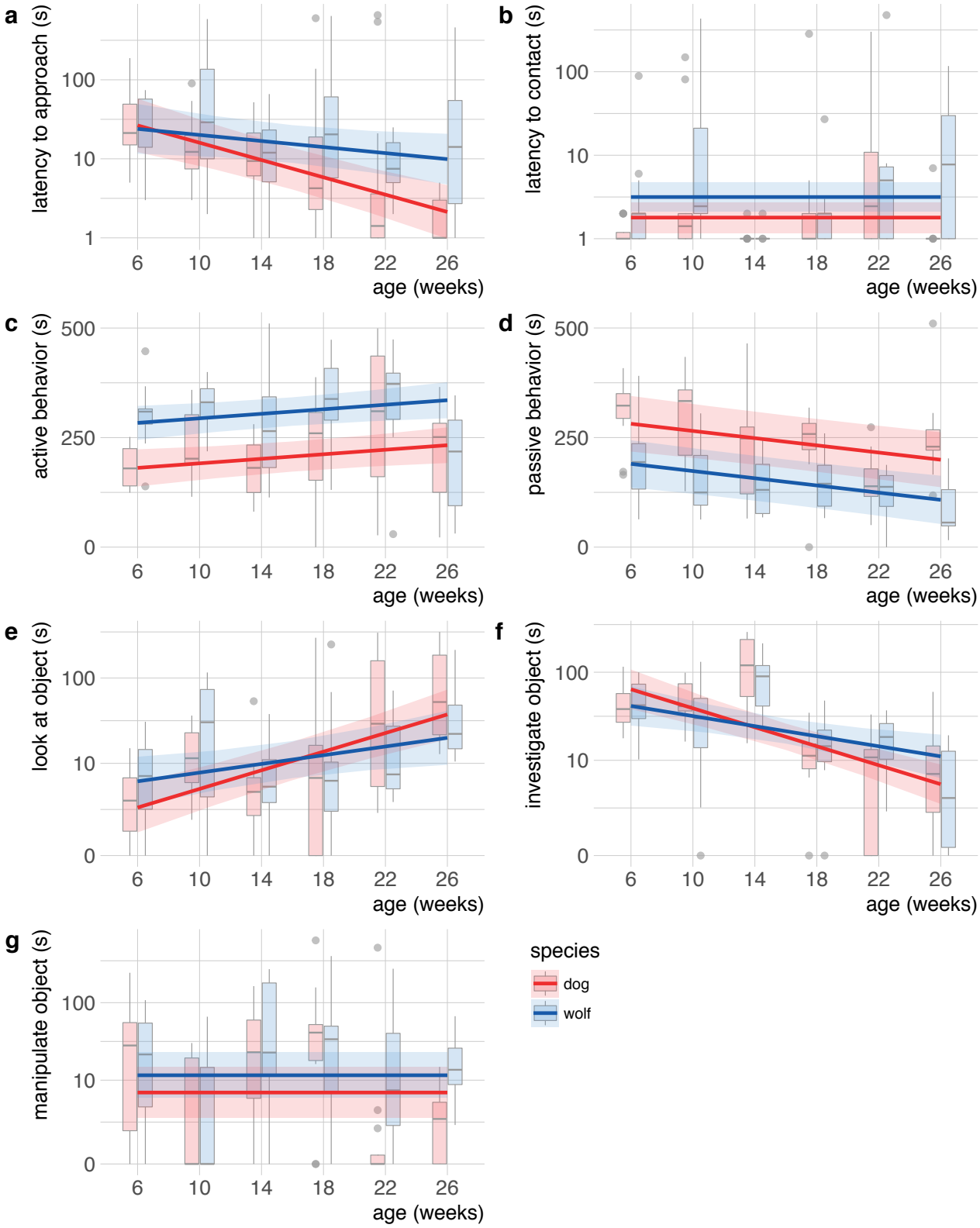
Table 2. Model summary. *Results for the best fitted model of repeated measures, with dogs as the reference, on 1) Latency to approach the novel object, 2) Latency to make contact with the novel object, 3) Looking at novel object (NO), 4) Investigating novel object, 5) Manipulating novel object, 6) Active behaviour and 7) Passive behaviour. Estimate, standard error, degress of freedom, t-value and p-value are given. Significant p-values are marked in bold italic.*

Behaviour	*Term*	*Estimate*	*Std. error*	*df*	*t*	*p*
Latency, approach	*(Intercept)*	0.875	0.133	2.240	6.576	***0.017***
	species	0.312	0.180	2.568	1.737	0.196
	age	-0.055	0.011	118.419	-5.067	***<0.0001***
	species:age	0.036	0.015	120.046	2.350	***0.020***
Latency, contact	*(Intercept)*	0.253	0.094	1.821	2.704	0.126
	species	0.246	0.129	2.164	1.913	0.186
Looking at NO	*(Intercept)*	2.136	0.827	94.332	2.581	***0.011***
	species	0.037	0.354	2.939	0.104	0.924
	age	0.121	0.021	117.899	5.848	***<0.0001***
	duration	0.001	0.001	124.417	0.426	0.671
	species:age	-0.064	0.031	120.667	-2.058	***0.042***
Invetsigating NO	*(Intercept)*	0.416	0.717	126.889	0.580	0.563
	species	0.141	0.234	3.493	0.601	0.585
	age	-0.119	0.019	138.727	-6.384	***<0.0001***
	duration	0.004	0.001	140.535	3.834	***0.0002***
	species:age	0.056	0.028	139.315	1.994	***0.048***
Manipulating NO	*(Intercept)*	2.812	1.078	90.449	2.610	***0.011***
	species	0.494	0.494	2.915	1.000	0.393
	duration	-0.001	0.002	141.262	-0.786	0.433
Active behavior	*(Intercept)*	-145.265	57.693	97.323	-2.518	***0.013***
	species	102.697	24.105	2.977	4.260	***0.024***
	age	2.587	1.176	122.299	2.200	***0.030***
	duration	0.598	0.090	127.554	6.644	***<0.0001***
Passive behavior	*(Intercept)*	152.645	54.263	25.688	2.813	***0.009***
	species	-91.478	39.977	3.003	-2.288	0.106
	age	-4.106	0.962	121.459	-4.268	***<0.0001***
	duration	0.149	0.074	127.572	2.029	***0.045***

Behaviours related to the novel object

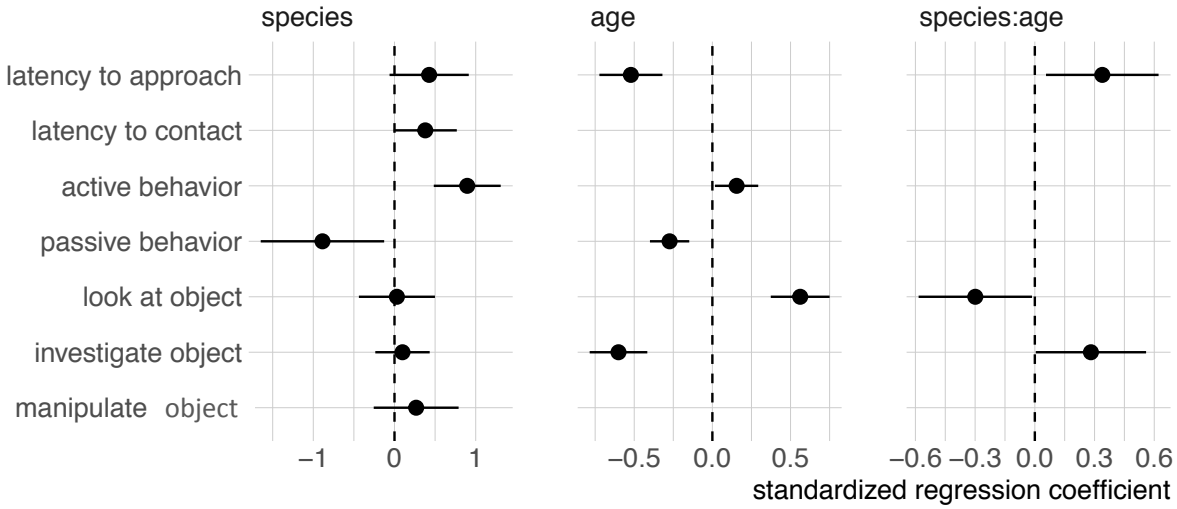
We found that wolves and dogs developed differently in looking at the novel object from a distance (t = -2.058, df = 120.667, p = 0.042, Table 2, Figure 1e), but no such differences were detected in the post hoc tests (Table S5). While both wolves and dogs increased their time spent looking at the novel object from a distance with age (t = 5.848, df = 117.899, p <0.001, Table 2, Figure 1e), dogs expressed a stronger effect of age than wolves (Figure 1e, Table S6).

Figure 1. Dog – wolf comparisons. *Boxplots shows behavioral scores during a novel object test, comparing dogs and wolves across age. Overlaid are the fits and confidence intervals from the best model, selected by AIC. Boxes indicate the quartiles, and the whiskers reach maximally 1.5 times the interquartile range. Values beyond that are shown as points. Note that panels a and b make use of a log(x) scale, and panels e, f and g use log(x + 1).*



Wolves and dogs also showed different developmental trajectories for the time spent investigating the novel object (t = 1.994, df = 139.315, p = 0.048, Table 2, Figure 1f and 2). Post-hoc tests revealed that wolves investigated the novel object for longer at 22 weeks than dogs (t = -2.831, df = 28.029, p = 0.008, $p_{adjusted}$ = 0.051, Figure 1f, Table S5). The significant interaction between species and age in investigating the novel object again consisted of stronger effect of age in dogs than in wolves (Figure 1f, Table S6), but with an overall decrease with age in both species (t = -6.384, df = 138.727, p <0.001, Table 2, Figure 1f). We found that wolves and dogs developed similarly in time spent manipulating the novel object (Table 2, Figure 1g). We found no evidence of sex differences.

Figure 2. Standardized regression coefficients*. Standardized regression coefficients. for the best model for each behavior, selected by AIC. Ranges indicate confidence intervals, computed using the likelihood profile. Missing estimates indicate that the term was not included in the best model.*



Behaviours not related to the novel object

We found that both species increased time spent on active behaviour with age (t = 2.2, df = 122.362, p = 0.03), with wolves expressing higher levels of activity than dogs (t = 4.26, df = 2.977, p = 0.024, Table 2, Figure 1c and 2, Table S5). Passive behaviour decreased with age in both wolves and dogs (t = -4.268, df = 121.140, p <0.001, Table

2, Figure 1d), and while dogs appeared more passive than wolves the species differences was not significant. We found no evidence of sex differences.

Discussion

Decreased expression of fear is considered a key behavioural alteration in domesticated animals, and evidence suggests that domestication drives altered developmental rates delaying the initial onset of fear response (2). However, while previous studies in wolves and dogs have focused exclusively on the timing of the initial onset of fear (23,30,31), it has been unclear if and how a developmental shift during early ontogeny, caused by domestication, affects the continued development and expression of fear in either species. Here we present the first extended examination of the development of fear behaviour within the juvenile period in wolves and dogs. Contrary to expectation, we found no evidence in support of a delayed onset of fear response in dogs compared to wolves during early stages of development. Instead we found that dogs strongly reduced their fear response to a novel object in the period between six and 26 weeks of age. Critically, wolves did not differ in their fear response towards novelty over time, and the detected species difference was caused solely by a progressive reduced fear response in dogs. Furthermore, dogs and wolves did on average not differ in their interaction with the novel object. Together our results suggest that species differences in fear of novelty are not caused by a domestication driven shift in the first onset of fear response. Instead, we suggest that a loss of sensitivity towards novelty with increasing age in dogs causes the difference in fear expression towards novelty in wolves and dogs.

Fearfulness has previously been quantified by the latency to approach and explore novelty, and novel stimuli such as objects, arenas and people have been used to

349 detect the timing of the initial onset of fear response in both wolves and dogs
350 (2,8,21,23,25,31) However, while there is a general expectation that domestication has
351 caused a delay in the sensitive period in dogs, resulting in later onset of fear
352 behaviour in dogs compared to wolves (8,22,30,31,47), we detected no species
353 differences in fear expression during early development. This finding is in
354 agreement with a recent study comparing exploration of novelty in six and eight
355 weeks old wolves and dogs, which found no species differences in the latency to
356 make contact with a novel object (32). Yet, it has been reported that adult wolves
357 express increased latency to make contact to a novel object compared to dogs (33),
358 thereby indicating that species differences in fear expression might arise later in
359 development than previously thought. Thus, our finding that a species difference in
360 latency to approach a novel object occurred at 26 weeks of age represents the first
361 indication of when a quantifiable difference in fear towards novelty arises in wolves
362 and dogs. We do, however, caution against an overly strong confidence in the exact
363 timing of species differences occurring at 26 weeks. It is possible that the difference
364 emerges in the weeks prior, but that the current sample size is insufficient for
365 detection. However, it is clear that a difference between species progressively
366 develops towards the later end of the time period measured here, and that we have
367 captured the transition from equal fear towards novelty to a clear species difference.
368 Importantly, the species difference in fear towards novelty did not occur because
369 wolves became more fearful with age, as expected, but rather because dogs
370 decreased their time to approach the novel object. Specifically, we detected no
371 increased expression of fear towards novelty in neither wolves nor dogs throughout
372 the study period, and notably wolves did not change their latency to approach the
373 novel object throughout with age. This indicates that the development of fear
374 response in wolves and dogs follows different trajectories, with dogs, but not
375 wolves, loosing their sensitivity towards novelty with age.

The species difference we found in the latency to approach the novel object is not clearly reflected in differences in interaction with the same novel object. While fear of novelty was expressed immediately, through a delayed approach, once the novel object was approached this initial fearfulness appears to no longer affect behavioural responses, and thus wolves and dogs did not differ in their latency to make contact with or interact with the novel object. While the latency to approach the novel object and the time spent being active and passive while in the test room showed consistent linear development over time in both wolves and dogs, the pattern in looking at, investigating and manipulating the novel object appeared variable across trials. This variability was most likely caused by the different novel objects that were used in the study, i.e. behaviours that are more closely related to the object itself show more variability across tests. For example, the stuffed wolverine toy clearly provided more incentive for manipulation than the upside-down wheelbarrow. It is possible that this increased variance may have prohibited detection of additional species differences in behavioural measures directly related to the novel object, such as increased exploration and manipulation of novel objects as reported in both juvenile and adult wolves compared to dogs (Moretti et al., 2015, Marshall-Pescini et al. 2017)

Wolves develop physically faster than dogs (48), and it has been suggested that wolves express increased activity at an earlier age than dogs due to this difference in developmental pace of motor patterns (32,48). However, while we do find a species difference in how much time is spent on active behaviour during tests, this species difference is consistent across age and not restricted to early ontogeny alone. This indicates that wolves, on a general scale, are more active than dogs. While it cannot be ruled out that active behaviour is affected by the presence of a novel object, it is a less likely explanation for our findings, as we measured behaviours in a non-overlapping way with priority of behaviours related to the novel object. Thus, the

measurement of activity does not include looking at, manipulating or approaching the novel object, but only time spent on active behaviour with no attention to the novel object. Instead the higher activity in wolves might reflect an increased reactivity of being separated from littermates and being confined in the test room compared to dogs.

Domestication has caused a general acceleration of sexual maturity in animals (12), and earlier sexual maturation in dogs (11,14) could explain the steeper behavioural change observed in dogs compared to wolves across some of the behaviours related to the novel object in our study. However, while reproduction in wild living wolf packs is restricted to the breeding couple, it is currently unclear if the lack of sexual activity in non-reproducing pack members is caused by delayed sexual maturity, behavioural suppression or restricted access to nutrition (49-51). Nevertheless, it has been demonstrated that captive wolves removed from social constraints sexually mature as early as nine months of age (50). Thus, it is unclear if we should expect behavioural ontogeny to be affected by a shift in developmental pace caused by earlier sexual maturity when comparing wolves and dogs living in captive, non-reproductive groups. Our study was conducted before sexual maturity occurred in either wolves or dogs and as we found no effect of sex on the expression of behaviour, we suggest that the steeper development of some behaviours in dogs are instead related to the loss of sensitivity towards novelty.

In conclusion, our study shows that wolves and dogs do not differ in their fear towards novelty before late in the juvenile phase. Importantly, the species difference does not occur because wolves become more fearful with age, but because dogs become less fearful with age. These findings have general implications for our interpretation of how domestication has shaped behavioural ontogeny. We suggest

that future studies quantify fear related behaviour on a long-term scale to increase our understanding of the behavioural trajectory of fear through different developmental stages.

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