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Breed differences in behavioural response to challenging situations in kittens

P. Marchei [a,*], S. Diverio [b], N. Falocci [c], J. Fatjó [a], J.L. Ruiz-de-la-Torre [a], X. Manteca [a]

[a] Departament de Ciència Animal i dels Aliments, Facultat de Veterinaria, Universitat Autonoma de Barcelona, 08193 Bellaterra, Spain
[b] Dpt. Scienze Biopatologiche ed Igiene delle Produzioni Animali e Alimentari, Facoltà di Medicina Veterinaria Via S. Costanzo 4, 06126 Perugia, Italy
[c] Servizio Studi, Consiglio Regionale dell'Umbria, Piazza Italia 2, 06121 Perugia, Italy

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ABSTRACT

In a previous experiment, the behaviour of Oriental/Siamese/Abyssinian (OSA) kittens was compared with that of Norwegian Forest kittens (NFO) in a repeated Open Field Test (OFT), and significant differences emerged. To further investigate such variations, we analyzed kittens' responses to a potentially threatening object (TO) during the OFT. It was a metal spring enveloped in a cotton case suddenly bouncing out of the cylinder after the first 6 min of OFT exposure, and the test lasted 6 more minutes. From the 4th to the 10th week of age, during each test, the response of 43 OSA kittens and 39 NFO kittens to the TO was analyzed. Heart rate (HR) before and after the test was recorded. Behaviours were recorded and analyzed by *focal animal sampling*. Behavioural modifications recorded after TO exposure confirmed our suggestions on slow limbic system development in NFO kittens, as previously suggested by poor habituation and poor memory retention of repeated OFT exposure. The evident avoiding response to the TO confirmed the adoption in NFO kittens of an active-coping strategy towards challenge, as indicated also by their high scores for exploration and escape attempts. Otherwise, poor TO influence on exploration observed in OSA kittens suggested the adoption of a passive coping strategy, as previously shown by low levels of exploration and intra-session reduction in the number of vocalizations. Nevertheless, some of the behaviours observed, and the evidence of emotional tachycardia in OSA kittens, suggested also that the low level of activity recorded could have been due to a low arousability predisposition in this breed. The perception of a poorly arousing potential in the experimental setting might have influenced the perception of danger and the behaviour adopted in OSA kittens.

1. Introduction

The Open Field Test (OFT) is a traditional test used in psychology for studying animal behaviour with a special emphasis on animal emotionality; the method was initially developed by Hall [1] for measuring spontaneous rodents' behaviour. It has then been used in several other species like rabbits, cats, avians and cows to measure emotional responses and behavioural differences on the basis of strains, breeds, rearing conditions, management and age [2–8]. In a previous research, the behaviour of Norwegian Forest (NFO) and Oriental, Siamese and Abyssinian (OSA) kittens, recorded from the 4th to the 10th week of age in an Open Field Arena containing a Novel Object (NO), was analyzed and compared among breeds and along time [9,10]. The experiment showed that kittens belonging to the two breed groups, irrespective of sex and although similar in age and housing, systematically differed in their behavioural response to the novel environment and to the NO. NFO kittens were more explorative and thigmotactic, whereas OSA kittens presented a precocious decrease of interest towards the OFT environment. Breed-related differences were explained through different hypothesis: (1) NFO kittens seem to be slower in development than OSA kittens; if so, they might not remember previous exposures to the arena and explore it as an unknown environment during each session; (2) although OSA and NFO kittens were selected to improve divergent morphological traits, some different behavioural and physiological traits might have been maintained or co-selected within each breed, making NFO kittens more likely to cope actively and OSA kittens to cope passively towards challenging situations. To further investigate the nature of the behavioural differences observed in kittens, we considered the strict relationship existing between approach/avoidance responses. In general, the natural behaviour of an animal exposed to a challenging situation is mainly driven by competition between environment-elicited exploration and aversion to novelty and potentially dangerous stimuli [11]. These responses are related to specific physiological and neuroendocrine mechanisms [12]. Also in humans it has been shown that people with greater anxiety levels present higher scores for approach–avoidance conflicts. In fact, as the conflict between curiosity and fear drives the responses to novelty, people with high anxiety-score avoid situations linked to curiosity [13]. Mice with low anxiety levels and high exploration scores present higher scores in inhibitory avoidance tasks [14]; animals that cope actively with novelty also tend to have an active behavioural strategy to cope with

* Corresponding author. Tel.: +39 328 9613234; fax: +39 075 5857654.
E-mail address: paola.marchei@uab.es (P. Marchei).

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aversive objects [15]. Notwithstanding the human deeper behavioural complexity, a similar feature has been observed in children: subjects reported to be reactive towards motivational-appetitive stimuli also tend to be reactive to motivational-aversive stimuli [16]. We supposed that also in cats, like in other species, traits like curiosity and anxiety should be successfully examined through the exam of approach/avoidance conflict response: to test our hypothesis, we analyzed the behavioural reaction to the potentially threatening object (TO) introduced during the 12 min lasting OFT, in order to investigate the relationship between novelty and the exposure to a potentially fear-eliciting item. Aim of the present study was to obtain complementary statistical information for evaluating if breed-related differences in behavioural responsiveness to novelty and treat were related to differences in neurological development, coping styles or to both mechanisms.

2. Materials and methods

2.1. Subjects

Thirty pure breed Oriental and Siamese kittens and thirteen Abyssinian kittens (OSA) and thirty nine Norwegian Forest kittens (NFO) from 12 breeders and 23 litters were studied; litters were born between June 2005 and July 2006; litter size varied from one to six kittens.

2.1.1. Housing and environment

The experiment was carried out in several catteries in the centre of Italy (Tuscany, Umbria and Lazio Regions). All kittens lived exclusively indoors in home environment in multi-cat household and had free access to water, food, litter-box and toys. The total number of cats in the household, except the litter and inclusive of the queen, varied from two to eleven. All breeders except one were married couples, they spent at least 1 h of time per day petting, cleaning, feeding the kittens and playing with them. All the kittens received visits and were manipulated by extraneous people at least once per week.

2.2. Physiological and behavioural sampling

2.2.1. Experimental design

Kittens were individually subjected to an Open Field Test (OFT) in their home environment. Each OFT was carried out between 9:00 am and 7:00 pm at the presence of the breeder. A total of 7 OFT were carried out at one week interval: the testing procedure started at the age of 4 weeks (mean age = 24.25 days, SD = 0.24 days) (OFT 1) and ended when the kitten reached the 10thweek of age (mean age = 65.55 days; SD = 0.40 days) (OFT 7). Details of the experimental calendar have been previously reported [10].

2.2.2. OFT arena structure

The OFT was performed in a room of the breeder's house where the experimenter could build up the Open Field arena. The arena was a white rounded box of 1.80 m diameter; the walls were 80 cm high compact polycarbonate layers; the floor was a 2 × 2 m nylon sheet divided into 30 cm area squares. Floor and walls of the OFT arena were cleaned with alcohol at the beginning of each test.

2.2.3. Novel object/aversive stimulus

The NO was a cylindrical metal container of 3.25 cm radius and 12 cm high fixed on a wooden slab 30 × 16 cm; it was placed inside the arena on the floor of the box, close to the wall, near the introducing site of the kitten. Reactions to novel environment and unknown object were measured for 6 min. When the kitten was in the same hemicircle where the NO was placed and looking at it, the potentially TO was introduced: the NO cylinder was suddenly opened provoking the abrupt exit of a 40 cm long metal spring enveloped in a cotton case, hidden inside the cylinder. The test lasted 6 more minutes.

2.2.4. Physiological data

At each age, the breeder removed a randomly chosen kitten from the nest, allowed the experimenter to measure baseline heart rate (HR-1) using a stethoscope (Littmann Classic II S.E., 3M™ Littmann®), then placed it into the OFT arena; after the OFT the breeder removed the kitten from the arena and allowed the experimenter to measure heart rate (HR-2) again. HR rate of response (HR-Resp) was calculated by dividing HR2 by HR1.

2.2.5. Behavioural data

Each OFT was recorded onto a videotape using a video camera (Sony Hi8 'Handycam') placed outside the arena on a 1.50 m high tripod. The kitten was placed in the arena by the breeder, on the opposite side of the same hemicircle where the video camera was placed, and left gently on the floor of the box near the wall. The OFT kitten's behaviour was observed during the two 6 min sessions using a *focal sampling* procedure. Behavioural measures included the number of vocalizations uttered and a range of behaviours grouped into categories, described in Table 1. Among these behaviours, accumulated times spent in each behaviour were measured as duration and expressed as seconds over the whole 360 s that represented each part of the test (FP – first part – before the exit of the spring; SP – second part – after the exit of the spring). Vocalizations were measured as frequencies and expressed as the number of times the behaviour was performed within the test.

2.3. Statistical analyses

All data were analyzed using SAS 9.1 Statistical Package (SAS Institute Inc. 2003, USA). Results of statistical analysis were considered significant at $p \leq 0.05$.

Effect of activity and locomotion on physiological values was evaluated using Spearman rank correlation coefficient, where activities implying locomotion were variables to correlate with HR-2 minus HR-1. The rate of increase in heart rate, calculated as HR2/HR1, was analyzed using two sample *t*-test.

Principal Component Analysis (PCA) was performed as a preliminary statistical exploration in order to evaluate any latent factor

Table 1
Description of behaviours observed during the OFT; all behaviours except vocalization were measured as durations; vocalizations were measured s frequencies.

Behaviour	Definition
Walking	Cat is walking or moving front legs or all four legs
Sitting	Cat is sitting back on haunches
Standing up	Cat is standing up on all four legs
Crouched	Cat is lying ventrally close to the floor in a compact position
Retreating	Cat is moving backwards
Walking and exploring walls	Cat is walking while sniffing the walls of the box
Walking and exploring floor	Cat is walking while sniffing the floor of the box
Resting and exploring walls	Cat is in a stationary position (sitting, standing or crouched) and sniffing the walls
Resting and exploring floor	Cat is in a stationary position (sitting, standing or crouched) and sniffing the floor
Exploring object	Cat is in contact with any part of the novel object, sniffing or touching it with nose, tongue or front paws
Rubbing object	Cat is rubbing any part of its body, usually head or lips on the novel object
Grooming	Cat is washing or scratching itself
Escape intents	Cat is scratching or climbing the wall, or is rearing with front paws on the wall
Other behaviour	Cat is performing a behaviour different from the described ones
Vocalization	Cat is uttering a "meow" call (all types of "meow")

associated to the whole behavioural pattern. Data were introduced in the model as mean values across animals to explore association between behaviours, breed, week and FP/SP.

A *Three way Analysis of Variance Model* (with repeated measurements) was performed in order to compare breed differences among FP/SP and weeks; the duration of a single behaviour was introduced as the dependent variable; categorical variables were breed, week and FP/SP and their interactions; the animal was considered as a random effect. Differences between weeks and between breeds were explored by Least Square Means χ^2 Test.

Finally, the *Generalized Random Effect Model* was used with two different specifications:

➢ *Gamma distribution,* in order to evaluate behavioural trends along time; the duration of a single behaviour was the dependent variable; week was considered as a covariate and not as factor; quadratic week term was included to asses the existence of non-linear behavioural trends along time. The model parameters are described above.

Parameter	Effect
$ß_1$	Breed
$ß_2$	Week
$ß_3$	Week^2
$ß_4$	Week*Breed
$ß_5$	Week*Breed^2

To improve text fluency, regression parameter coefficients of explanatory variables are indicated only with the corresponding Beta symbols.

➢ *Binomial distribution,* in order to estimate breed and FP/SP dependent changes in the probability of a behaviour to be performed; the dependent variable was the presence (1) or absence (0) of the behaviour; breed and FP/SP were categorical variables.

3. Results

3.1. Physiological data

Throughout the whole experimental period, HR-2 was significantly higher than HR-1 in both breeds (OSA: $t\ (293) = -8.23$, $p < 0.001$; NFO: $t\ (320) = -9.08$, $p < 0.001$). No statistical differences emerged in HR-Resp among breeds, but the value was almost higher in NFO kittens ($t\ (256) = 1.91$, $p = 0.057$) and mean HR-Resp was higher in NFO (1.59) than in OSA (1.47) kittens (Fig. 1). No positive correlation existed between rise in HR and locomotor activity during the test, so any increase in HR was considered as induced by stress.

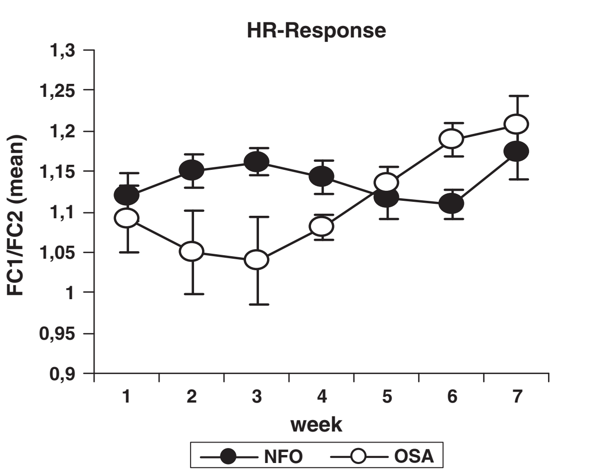

Fig. 1. Mean HR-Response (±S.E.) observed in Norwegian (NFO) and in Oriental/Siamese/Abyssinian (OSA) kittens weekly exposed to the OFT.

3.2. Behavioural data

The *Principal Component Analysis* indicated two components explaining over 90% of the behavioural patterns observed; Component 1 could be defined as "anxiety"; the axis distribution evidenced "anxious" behaviours like *crouched* and *escape attempts* on one extreme and "relaxed" behaviours, like *walking and exploring floor* on the other extreme. Anxious behaviours appeared on the same plot side of SP in both breeds, suggesting that the TO was actually frightening for the kittens. NFO kittens matched with *escape attempts*, OSA kittens with the *crouched* posture. Component 2 seemed to be linked to "interest in exploration" or "curiosity", as from the higher to the lower values of the axis there was a regular occurrence of behaviours denoting increasing curiosity towards surroundings. Curiosity-denoting behaviours were on the same side of the plot of NFO kittens during FP and SP (Fig. 2).

3.2.1. NO exploration

The *latency to explore the NO* presented a parabolic change in both breeds, with the shorter latency recorded in central weeks (FP: $ß_3 = 11.63$; $p = 0.0006$; SP: $ß_3 = 4.17$; $p = 0.0412$); nevertheless, only in NFO kittens the latency increased after the TO ($\chi^2(1) = 6.43$, $p = 0.0112$). During the first contact, NFO kittens spent more time than OSA kittens exploring the NO ($\chi^2(1) = 5.36$, $p = 0.0206$) but in OSA kittens this score increased after the introduction of the TO ($\chi^2(1) = 12.29$, $p = 0.0005$) (Fig. 3; Table 2). In general, both breeds explored the NO longer in SP than in FP (OSA: $\chi^2(1) = 10.47$, $p = 0.0012$; NFO: $\chi^2(1) = 86.17$, $p = 0.013$) but after the TO in the NFO kittens the probability of performing this behaviour decreased ($\chi^2(1) = 9.22$, $p = 0.0024$). Both breeds spent more time *rubbing* on the object during SP ($\chi^2(1) = 4.24$, $p = 0.0396$). However, during SP the probability increased in OSA kittens ($\chi^2(1) = 12.91$, $p = 0.0003$), while in NFO kittens decreased ($\chi^2(1) = 7.22$, $p = 0.076$). Breed-related behavioural differences observed in kittens' approach towards the NO/TO are summarized in Table 3.

3.2.2. Changes in locomotion and resting postures scores

3.2.2.1. Locomotion. The time spent *walking* among weeks presented a parabolic change in FP ($ß_3 = 5.10$; $p = 0.024$) and all kittens spent

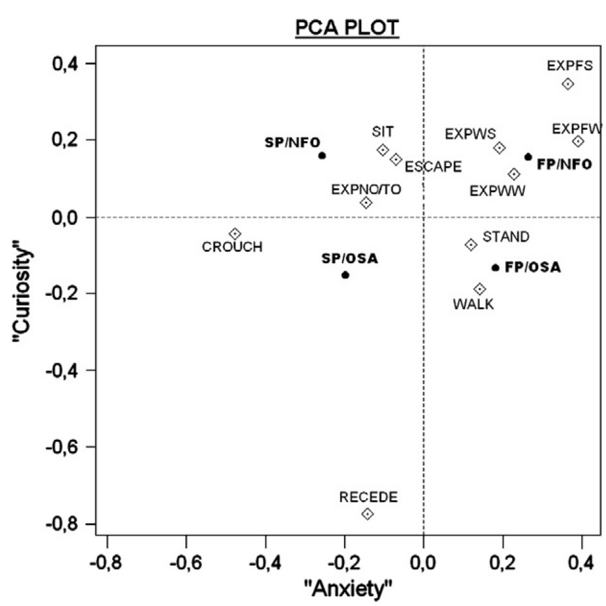

Fig. 2. PCA Plot; SIT (Sitting); CROUCH (Crouched); EXPNO/TO (Exploring NO/TO); recede (Retreating); WALK (Walking); STAND (Standing); EXPWW (Exploring walls while walking); ESCAPE (Escape attempts); EXPWS (Exploring walls while standing); EXPFW (Exploring floor while walking); and EXFS (Exploring floor while standing).

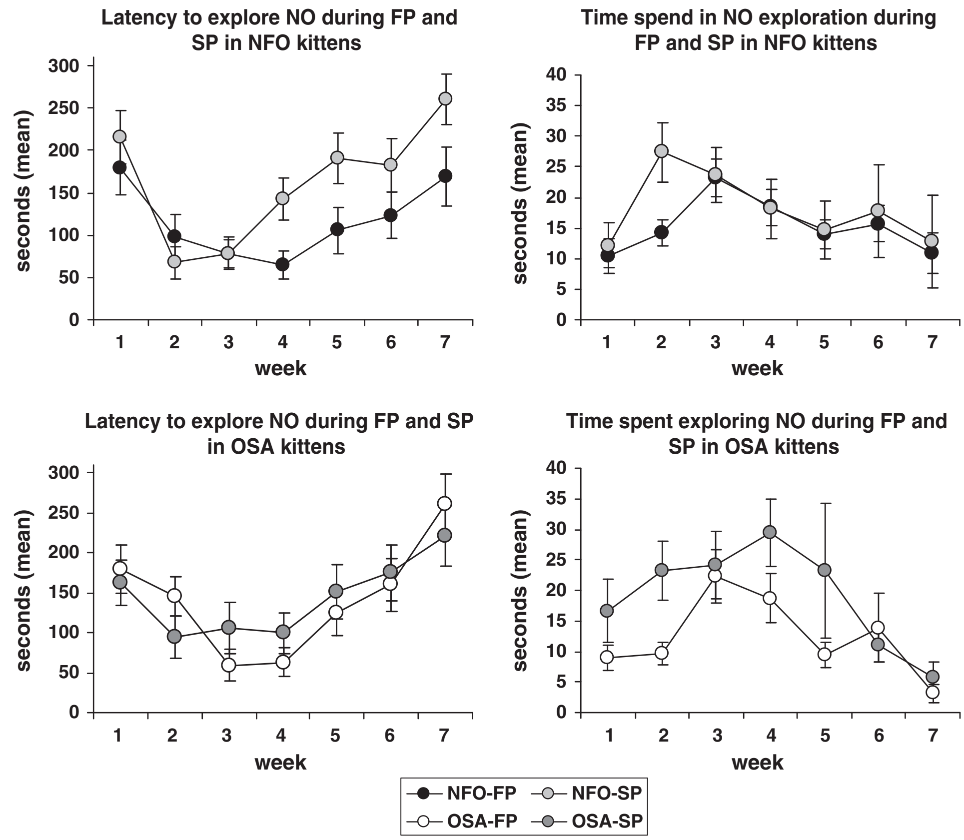

Fig. 3. Open Field behaviour of Norwegian (NFO) and Oriental/Siamese/Abyssinian (OSA) kittens. Data for both groups are merged to show weekly evolution for the latency to explore NO and for the time spent exploring it during FP Oriental/Siamese/Abyssinian (First Part of the Test) and SP (Second Part of the test). Values are expressed as seconds ± S.E over each OFT session (360 s).

less time *walking* after the introduction of the TO ($\chi^2(1)=39.68$ $p<0.0001$) (Fig. 4; Table 4); OSA kittens always spent more time than NFO kittens *walking* ($\chi^2(1)=9.49$ $p=0.0021$) and *retreating* (FP: $\chi^2(1)=15.53$, $p<0.0001$; SP: $\chi^2(1)=16.15$, $p<0.0001$); in NFO kittens the time spent *retreating* increased in SP ($\chi^2(1)=8.76$, $p=0.0031$) while no change was observed in OSA kittens (Fig. 4). In both breeds the TO increases the probability that animals retreated ($\chi^2(1)=28.22$, $p<0.0001$), but such probability increases more in NFO kittens ($\chi^2(1)=22.29$, $p<0.0001$) than in OSA kittens ($\chi^2(1)=6.69$, $p=0.0097$).

3.2.2.2. Resting postures. During SP, all kittens passed less time *standing* (NFO: $\chi^2(1)=20.35$, $p<0.0001$; OSA: $\chi^2(1)=4.40$ $p<0.0359$), but NFO kittens spent more time than OSA kittens in this behaviour ($\chi^2(1)=4.09$, $p=0.0431$). The behaviour presented a lineal decline from the 1st to the last week of observation (FP: $ß_2=52.45$, $p=0.0001$; SP: $ß_2=38.23$, $p<0.0001$). The behaviour *sitting* increased along time during FP ($ß_2=11.52$, $p=0.0007$) in both breeds, and only in NFO kittens during SP ($ß_3=4.24$, $p<0.0396$). NFO kittens were always more likely to stay sitting than OSA kittens (FP: $\chi^2(1)=12.65$,

Table 2
NFO and OSA weekly significant differences in *latency time to contact NO* during SP. Estimated values take the unit of measure of the corresponding studied parameter (seconds).

NFO weekly differences in latency time to contact NO during SP					
Week vs week		Estimated value	SE	χ^2	*p value*
1st	2nd	0.0261	5.60	5.60	0.0179
1st	3rd	0.0246	8.48	8.48	0.0036
2nd	5th	0.0214	5.31	5.31	0.0212
2nd	6th	0.0248	4.27	4.27	0.0387
2nd	7th	0.0498	6.27	6.27	0.0095
3rd	5th	0.0218	7.45	7.45	0.0064
3rd	6th	0.0252	6.13	6.13	0.0133
3rd	7th	0.0500	9.58	9.58	0.0020
4th	7th	0.0510	7.08	7.08	0.0078
OSA weekly differences in latency time to contact NO during SP					
Week vs week		Estimated value	SE	χ^2	*p value*
4th	7th	−0.1416	0.0730	5.61	0.0179

Table 3
Estimated significant differences in the time spent in behaviours related to NO/TO approach after the introduction of the threatening stimulus. Estimated values take the unit of measure of the corresponding studied parameter (seconds or number of animals).

Estimated differences in time spent in NO explorative behaviours after introduction of the threatening stimulus					
Explorative behaviours	Breed	Estimated value	Standard error	χ^2	*p value*
Latency to explore NO	NFO	0.0025	0.0010	6.43	0.011
	OSA	0.0001	0.0007	0.05	0.832
Time spent exploring NO at first contact	NFO	0.0251	0.0442	0.32	0.570
	OSA	0.1551	0.0443	12.30	< 0.001
Total time spent exploring NO	NFO	0.0089	0.0039	6.17	0.013
	OSA	0.0270	0.0087	9.62	0.0019
Rubbing on the NO	NFO	0.5672	0.6251	0.82	0.364
	OSA	2.1649	1.1898	3.31	0.068
Number of kitten exploring NO	NFO	−0.8329	0.2743	9.22	0.024
	OSA	−0.0603	0.2131	0.08	0.775
Number of kitten rubbing on the NO	NFO	0.1921	0.1856	1.11	0.293
	OSA	0.9230	0.2569	12.91	0.003

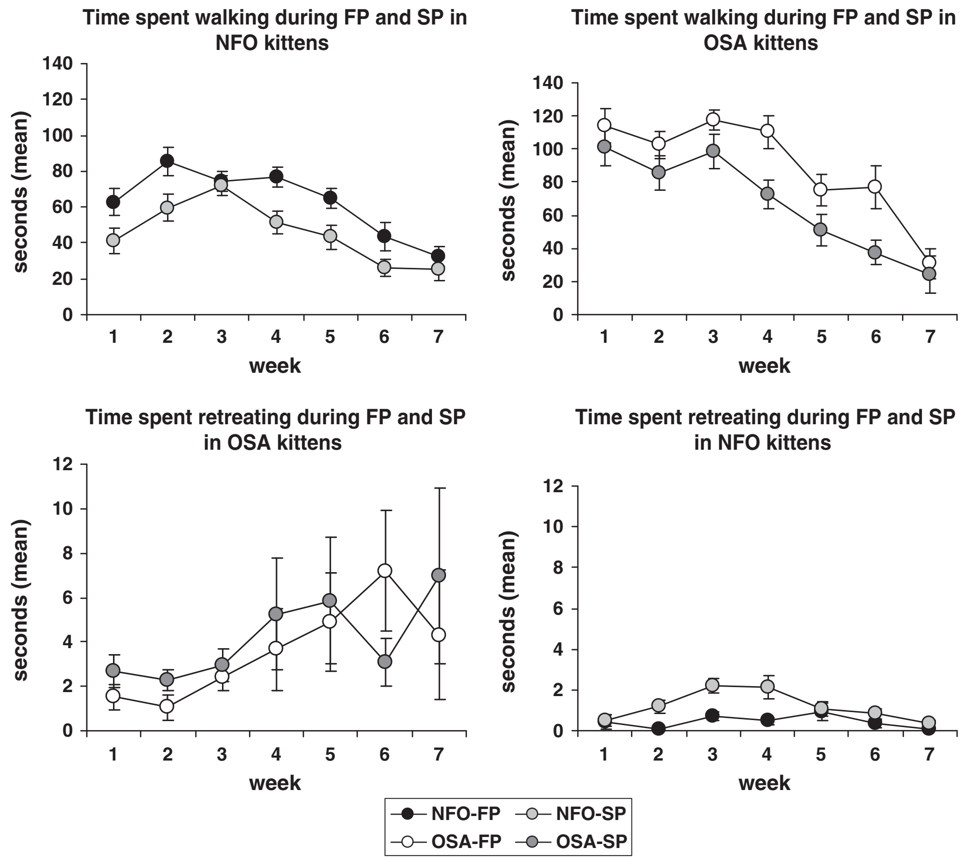

Fig. 4. Open Field behaviour of Norwegian (NFO) and Oriental/Siamese/Abyssinian (OSA) kittens. Data for both groups are merged to show weekly evolution for time spent walking and retreating during FP (First Part of the Test) and SP (Second Part of the test). Values are expressed as seconds ± S.E over each OFT session (360 s).

$p = 0.0004$; SP: $\chi^2(1) = 14.82$, $p = 0.0001$). After the introduction of the TO, there was a lineal increase in the time spent *crouched* along time ($ß_2 = 4.25$, $p = 0.0393$); compared to FP, in both breeds there was an increase in the time spent *crouched* (NFO: $\chi^2(1) = 11.33$, $p = 0.0008$; OSA: $\chi^2(1) = 6.88$, $p = 0.0087$) and in the probability of performing the behaviour (NFO: $\chi^2(1) = 30.89$, $p < 0.0001$; OSA: $\chi^2(1) = 21.51$, $p < 0.0001$). The probability of staying *crouched* in NFO was always higher than in OSA kittens ($\chi^2(1) = 8.14$, $p < 0.0043$).

3.2.3. Changes in exploratory behaviour

Only NFO kittens reduced the time spent *walking and exploring wall* after the TO ($\chi^2(1) = 20.86$, $p < 0.0001$). Along time there was a parabolic change in the time spent *walking and exploring wall*, with the maximum values during central weeks of observations (FP: $ß_3 = 14.04$; $p = 0.0002$; SP: $ß_3 = 8.59$, $p = 0.0034$). During FP, NFO kittens spent more time than OSA kittens exploring walls while *walking* ($\chi^2(1) = 4.39$, $p = 0.0367$) and *standing* ($\chi^2(1) = 10.65$, $p = 0.0011$) The probability of NFO kittens performing the latter behaviour in FP was higher ($\chi^2(1) = 10.49$, $p = 0.0012$), but presented a reduction in SP ($\chi^2(1) = 9.52$, $p = 0.002$). In both breeds the time spent *standing and exploring walls* decreased after the TO (NFO: $\chi^2(1) = 18.64$, $p < 0.0001$; OSA: $\chi^2(1) = 5.633$, $p = 0.0177$) but presented a parabolic change, with the maximum values observed in central weeks, in both sessions (FP: $ß_3 = 5.95$; $p = 0.0147$; SP: $ß_3 = 3.94$, $p = 0.0472$) (Fig. 5; Table 5).

In SP there was a decrease in the time spent *walking and exploring floor* (NFO: $\chi^2(1) = 41.73$, $p < 0.0001$; OSA: $\chi^2(1) = 9.20$, $p = 0.0024$) and in the probability of performing the behaviour *exploring floor while walking* (NFO: $\chi^2(1) = 33.89$, $p < 0.0001$; OSA: $\chi^2(1) = 13.63$, $p = 0.0002$) (Fig. 5; Table 6). NFO kittens spent more time than OSA kittens *walking and exploring floor* only during FP ($\chi^2(1) = 5.24$, $p = 0.0221$) but the probability of performing the behaviour was always higher in NFO kittens (FP: $\chi^2(1) = 7.73$, $p = 0.0054$; SP: $\chi^2(1) = 3.96$, $p = 0.0467$).

All kittens spent more time *standing and exploring floor* in FP (NFO: $\chi^2(1) = 40.66$, $p < 0.0001$; OSA: $\chi^2(1) = 17.79$, $p < 0.001$) and the probability that kittens performed the behaviour in SP decreased ($\chi^2(1) = 24.70$, $p < 0.0001$). NFO kittens always spent more time than OSA kittens *standing and exploring floor* (FP: $\chi^2(1) = 8.30$, $p = 0.0040$; SP: $\chi^2(1) = 10.68$, $p = 0.0011$) and the probability that NFO performed the behaviour was always higher in NFO than OSA kittens (FP: $\chi^2(1) = 14.77$, $p = 0.0001$; SP: $\chi^2(1) = 18.69$, $p < 0.0001$) (Fig. 5; Table 7).

Table 4
NFO and OSA weekly significant change in time spent walking from FP to SP. Estimated values take the unit of measure of the corresponding studied parameter (seconds).

NFO weekly differences in time spent walking from FP to SP				
Week	Estimated value	SE	χ^2	*p value*
1st	−0.0084	0.0035	5.66	0.0174
2nd	−0.0051	0.0020	6.82	0.0090
4th	−0.0064	0.0019	11.24	0.0008
5th	−0.0095	0.0032	8.53	0.0035
6th	−0.0154	0.0049	9.75	0.0018
OSA weekly differences in time spent walking from FP to SP				
Week	Estimated value	SE	χ^2	*p value*
4th	−0.0047	0.0015	9.58	0.0020
5th	−0.0053	0.0026	4.26	0.0391
6th	−0.0136	0.0046	8.76	0.0031

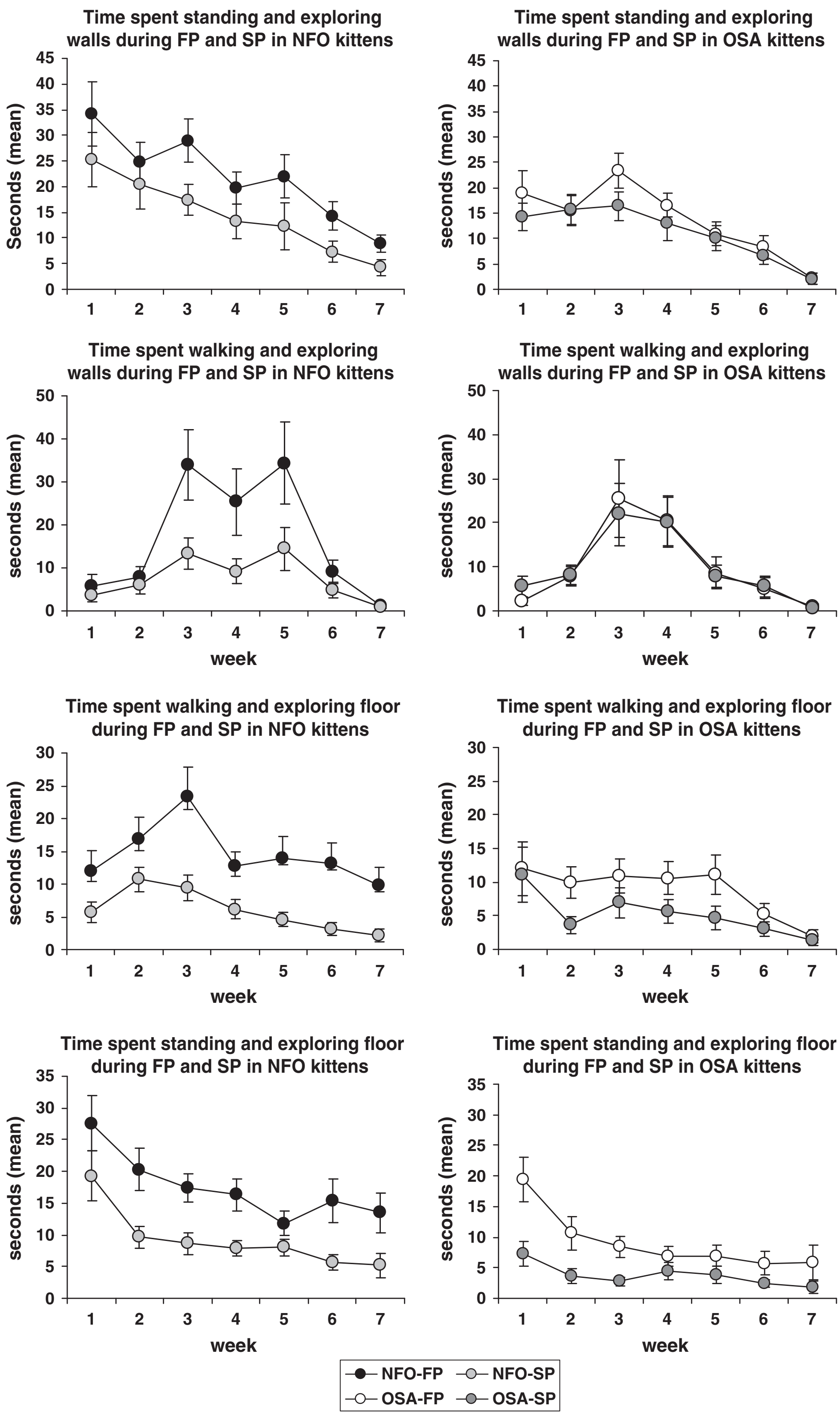

Fig. 5. Open Field behaviour of Norwegian (NFO) and Oriental/Siamese/Abyssinian (OSA) kittens. Data for both groups are merged to show weekly evolution for the time spent exploring walls and floor while standing and walking during FP (First Part of the Test) and SP (Second Part of the test). Values are expressed as seconds ± S.E over each OFT session (360 s).

3.2.4. Escape attempts

In OSA kittens, no differences emerged in the time spent in *escape attempts* between FP and SP. In NFO kittens the time spent in escape attempts was almost higher during SP ($\chi^2(1) = 3.65$, $p = 0.0562$). In both breeds there was a lineal increase along weeks in the time spent in escape attempts (FP: $ß_2 = 6.03$, $p = 0.0140$; SP: $ß_2 = 4.54$, $p = 0.0331$); during SP, NFO spent more time than OSA kittens in this behaviour ($\chi^2(1) = 4.47$, $p = 0.0346$) and the probability that OSA

Table 5
NFO and OSA weekly significant differences in time spent standing and exploring walls during FP and SP. Estimated values take the unit of measure of the corresponding studied parameter (seconds).

NFO weekly significant differences in time spent standing and exploring walls during FP

Week vs week		Estimated value	SE	χ^2	*p value*
1st	4th	−0.0214	0.0085	6.31	0.0120
1st	6th	−0.0408	0.0144	8.01	0.0046
1st	7th	−0.0843	0.0208	16.48	<0.0001
2nd	6th	−0.0297	0.0149	3.33	0.0457
2nd	7th	−0.0732	0.0233	9.86	0.0017
3rd	4th	−0.0161	0.0064	6.26	0.0123
3rd	6th	−0.0355	0.0126	7.99	0.0047
3rd	7th	−0.0790	0.0200	15.53	<0.0001
4th	7th	−0.0628	0.0210	8.91	0.0028
5th	6th	−0.0265	0.0134	3.94	0.0471
5th	7th	−0.0700	0.0209	11.25	0.0008
6th	7th	−0.0435	0.0175	6.18	0.0129

NFO weekly significant differences in time spent standing and exploring walls during SP

Week vs week		Estimated value	SE	χ^2	*p value*
1st	4th	−0.0360	0.0161	4.98	0.0257
1st	6th	−0.0980	0.0386	6.52	0.0107
1st	7th	−0.1971	0.0859	5.27	0.0217
2nd	6th	−0.0890	0.0412	6.41	0.0114
2nd	7th	−0.1877	0.0871	4.64	0.0312
3rd	6th	−0.0806	0.0369	4.79	0.0287
3rd	7th	−0.1793	0.0839	4.56	0.0327

OSA weekly significant differences in time spent standing and exploring walls during FP

Week vs week		Estimated value	SE	χ^2	*p value*
3rd	4th	−0.0174	0.0087	3.97	0.0464
3rd	5th	−0.0529	0.0221	5.72	0.0168
3rd	6th	−0.0775	0.0321	5.84	0.0157

OSA weekly significant differences in time spent standing and exploring walls during SP

Week vs week		Estimated value	SE	χ^2	*p value*
1st	6th	−0.0823	0.0416	3.91	0.0481
2nd	6th	−0.0889	0.0415	4.60	0.0320
3rd	6th	−0.0913	0.0399	5.24	0.0221

kittens tried to escape decreased ($\chi^2(1) = 4.65$, $p = 0.031$), whereas in NFO kittens the probability of performing this behaviour did not change.

3.2.5. Vocalizations

In general, OSA kittens vocalized more than NFO kittens only during FP ($\chi^2(1) = 11.08$, $p = 0.0009$); in OSA kittens there was a decrease in the number of vocalizations uttered from FP to SP ($\chi^2(1) = 9.04$, $p = 0.0026$) that was lacking in NFO kittens.

4. Discussion

4.1. Development of memory

As described in our previous study [9], the gradual decrease in locomotion scores and the increase in time spent in inactive behaviours within session and among weeks can be linked to the normal tendency of animals to reduce, after initial exploration, the level of activity in a novel environment [17]. We also suggested a differential neurological development in the two breeds: OSA kittens' higher locomotion levels and NFO kittens' increase in non-weight bearing postures could indicate a faster neuromuscular development in the former breed [18]; the supposed limbic system immaturity in NFO

Table 6
NFO and OSA weekly significant differences in time spent walking and exploring floor during FP and SP. Estimated values take the unit of measure of the corresponding studied parameter (seconds).

NFO weekly significant differences in time spent walking and exploring floor during FP

Week vs week		Estimated value	SE	χ^2	*p value*
1st	3rd	0.0406	0.0203	3.99	0.0458
3rd	4th	−0.0358	0.0140	6.55	0.0105

NFO weekly significant differences in time spent walking and exploring floor during SP

Week vs week		Estimated value	SE	χ^2	*p value*
2nd	5th	−0.1301	0.0590	4.86	0.0275
2nd	6th	−0.2231	0.1031	4.68	0.0305
3rd	5th	−0.1165	0.0551	4.47	0.0344
3rd	6th	−0.2095	0.1020	4.22	0.0399

OSA weekly significant differences in time spent walking and exploring floor during FP

Week vs week		Estimated value	SE	χ^2	*p value*
4th	6th	−0.0922	0.0459	4.04	0.0444
5th	6th	−0.0945	0.0477	3.92	0.0476

OSA weekly significant differences in time spent walking and exploring floor during SP

Week vs week		Estimated value	SE	χ^2	*p value*
1st	2nd	−0.1843	0.0934	3.90	0.0483
1st	6th	−0.2374	0.1131	4.41	0.0358

Table 7
NFO and OSA weekly significant differences in time spent standing and exploring floor during FP and SP. Estimated values take the unit of measure of the corresponding studied parameter (seconds).

NFO weekly significant differences in time spent standing and exploring floor during FP

Week vs week		χ^2	SE	Estimated value	*p value*
1st	3rd	5.83	0.0088	−0.0213	0.0158
1st	4th	5.90	0.0103	−0.0251	0.0151
1st	5th	10.52	0.0141	−0.0457	0.0012
1st	7th	4.92	0.0171	−0.0380	0.0266

NFO weekly significant differences in time spent standing and exploring floor during SP

Week vs week		χ^2	SE	Estimated value	*p value*
1st	2nd	6.13	0.0210	−0.0519	0.0133
1st	3rd	6.00	0.0263	−0.0644	0.0143
1st	4th	10.12	0.0232	−0.0738	0.0015
1st	5th	14.13	0.0189	−0.0709	0.0002
1st	6th	11.78	0.0355	−0.1217	0.0006
1st	7th	4.20	0.0691	−0.1416	0.0404
2nd	5th	5.22	0.0276	−0.0190	0.0226

OSA weekly significant differences in time spent standing and exploring floor during FP

Week vs week		χ^2	SE	Estimated value	*p value*
1st	2nd	3.88	0.0213	−0.0420	0.0489
1st	3rd	8.63	0.0226	−0.0663	0.0033
1st	4th	7.18	0.0350	−0.0939	0.0074
1st	5th	8.31	0.0332	−0.0958	0.0039
1st	6th	4.81	0.0172	−0.1222	0.0283

OSA weekly significant differences in time spent standing and exploring floor during SP

Week vs week		χ^2	SE	Estimated value	*p value*
1st	3rd	6.41	0.0883	−0.2237	0.0113
1st	6th	5.78	0.1104	−0.2654	0.0162

kittens could explain poor memory retention of previous exposures and thus poor habituation to the OFT [2,9,12,17,19]. Moreover, results obtained analyzing the response to the TO showed that it had a strong influence on their behaviour along all the experimental period. This data confirmed our hypothesis, as repeated exposures to an aversive stimulus should elicit the higher fear-related emotional response in correspondence of the initial exposure; then the response should decrease with habituation [11,20] unless memory neural pathways are still immature. Although in OSA and NFO kittens there was a logical modulation of behaviours along time, indicating memory retention of previous exposures in both breeds, NFO kittens showed little habituation to the OFT arena and to the TO notwithstanding the high exploration scores. This finding could be explained by some recent investigations on rodents: it has been shown that learning abilities and memory retention in mice are strongly influenced by the ability of an animal to dedicate selective attention to relevant cues while ignoring distractions [21] so an highly explorative animal might not be necessarily able to focus on the important stimuli during general environment exploration. Finally, an investigation carried out on mice exposed to several learning tasks showed that sensitivity to stress, exploratory tendency and general learning ability are not necessarily linked one to each other [22]: in that experiment animals treated with anxiolytic drugs showed less propensity to explore unwalled quadrants in the OFT; lower stress reactivity and reduced corticosterone response didn't seem to produce enhancement in learning abilities during the performed tests [22].

4.2. Coping strategies

Low behavioural flexibility and poor acceptance of new environment are typical features of active-coping animals [23,24]. Indeed, high exploration scores in NFO kittens suggest an active strategy to cope with novelty as well as a lower stress level in the arena [9], in agreement with previous studies [23–26]. NFO kittens clearly have an avoiding response to the TO, confirmed by the higher levels of behaviours like retreating and escape attempts. The increase in the number of kittens that do not approach the TO at all is consistent with results in rodents, where active behaviours towards novelty are accompanied by a higher latency to approach aversive stimuli [14]. According to literature [23,24] this pattern of reaction would be accompanied by low pituitary-adrenal axis (PAA) reactivity and high sympathetic reactivity. On the other side, OSA kittens low level of exploration denotes a passive strategy towards challenging situations, as well as a higher level of stress that impairs investigation of the environment: the introduction of the TO decreases the level of activity but only slightly influences exploration. In SP the number of kittens that try to escape decreases and they also tend to reduce the amount of distress cries uttered. We already recognised in such behavioural traits a passive coping strategy [9], but it should be accompanied by high PAA and parasympathetic reactivity and low sympathetic reactivity [15,22,23,25,26]. Interestingly, whereas reactive animals are expected to present, in reaction to a sudden unpredicted stressor, a bradycardia response induced by a prevalence of the parasympathetic system [27], we observed emotional tachycardia in both breeds, even if the HR-Resp was slightly smaller in OSA kittens. One explication for such contradictory detection could be related to the different sympathetic system activation in cats, compared to other species: in fact, even if few studies investigated cats neuroendocrinological response to fearful stimuli, it has been clearly shown that cat's locus coeruleus (LC) neurons do not appear to play a specific role in cardiovascular regulation and, while they generally co-activate with the sympathetic nervous system in other species, in cats they can be dissociated [28]. The LC of the cat deeply differs from that of rodents and primates, both in its anatomy and in its sensitivity to external stimuli: LC discharge rates during alert waking in cats are lower than that of rats and monkeys. In the latter species, arousal-increasing stimuli elicit tonic discharge higher than those elicited in cats, and novel non-stressful stimuli don't elicit phasic discharges in cat LC neurons at all [29–31].

4.3. Arousal potential of the OFT

The divergent effect of the potentially fearful stimulus in the two breeds could be explained both with the hypothesis of divergent coping strategies as well as with the idea of a faster development of memory in OSA kittens: more pronounced learning among sessions might reduce their response toward the TO, and their passive coping strategy would make those kittens more prone to accept the environment. A confounding feature is that OSA adult cats are commonly recognised by breeders and veterinarians to be exceptionally active, curious and interested towards novelty and their surrounding. The adaptation strategy that promotes fitness during a given ontogenetic stage might not be functional later in time [32,33], so a passive cope during early stages of life can be followed in adulthood by an active strategy toward challenging situations [34,35]. Our findings could also be explained with a differential susceptibility to arousing stimuli and different perception of challenge in NFO and OSA kittens.

Previous investigations on natural defensive behaviour, stressors and aversive behavioural responses in cats showed that the magnitude of the response depends on elements like movement, novelty and physical distance, and on the arousal potential of the stimulus, which is fundamental for generating, in the subject to which it is directed, an high arousal level, intended as alertness and activation [36,37]. So a moving object appears more threatening than an immobile object, and the unexpected apparition of it, as well as its proximity, increases the level of fear induced [20,38–40]. On the other side, investigations carried out on the arousal potential of a stimulus indicate that the extent to which a stimulus is capable of raising arousal depends on (1) physical properties like loudness, colour and speed; (2) ecological properties, related to survival, like fear and danger; (3) collative properties such as novelty, complexity or incongruity [37,41]. Such properties are processed by the individual, and the mode of perceptual processing of the stimulus is related to personality traits [37,41]. The arena test environment seems to be a high arousing setting for NFO kittens, as documented by their behavioural reactions, whereas OSA kittens might perceive in such environment a high threshold for arousal. This supposition would be confirmed in the latter breed by the low general level of exploration and the poor influence of the TO in the overall behaviour. After the introduction of the TO, both breeds spend more time exploring and rubbing on it; rubbing behaviour has been shown to be positively correlated with high arousal level and active behaviours like grooming, walking and interest in food [42–44]. Thereafter, because of the properties described above, the TO might be not only more threatening than the NO, but might also present: a higher arousal potential (production of noise during opening, movement of the spring, more complex appearance). Interestingly, only in OSA kittens the number of animals that rub on the NO is higher after the exit of the spring from the cylinder, and the time spent exploring it at the first contact increases, suggesting that they need a higher arousal potential to be aroused. In fact, after NO opening in SP, the number of NFO kittens exploring it decreases. However, these NFO subjects showing interest in NO also spend a longer time rubbing on it. This suggests that, in FP, the majority of NFO kittens has already spent enough time exploring the NO and only a minority shows a renewed interest in it after the NO opening.

Finally, heart rate increase is used in other species as a standard index for the level of Central Nervous System activation, anxiety and alertness towards fear-arousing events [8,36,41,45,46]. The lower HR-Resp observed in OSA kittens, together with behavioural findings, would suggest that these animals present a higher arousability threshold.

5. Conclusions

Analysis of the reactions towards the threatening object in the OFT turned out to be a valuable tool for clarifying the nature of previously observed differences in the development of growing kittens and differences in the emotional response during the early stages of life. Divergent patterns of behaviour observed in NFO and OSA kittens are very likely to depend on a combination of different neurological development among breeds with a different strategy towards challenge. Nevertheless, the inconsistency observed between the behavioural coping strategies and the physiological findings suggests that physiological features that typically accompany behavioural response to challenge in other species can't be easily extrapolated to the cat. On the other side, some behavioural detections and the evidence of emotional tachycardia also in OSA kittens, seem to suggest that the passivity of those animals in our experimental design could be due not only to a passive coping strategy but also to a lower arousing potential of the whole experimental setting in this breed. So the different arousability predisposition might influence the perception of danger in the arena test and, consequently, the coping strategy adopted. The use of anxiety-reducing drugs, as well as behaviour activating compounds in the kittens, might help to elucidate this hypothesis.

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