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Maternal predator-exposure has lifelong consequences for offspring learning in threespined sticklebacks

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Learning is an important form of phenotypic plasticity that allows organisms to adjust their behaviour to the environment. An individual's learning performance can be affected by its mother's environment. For example, mothers exposed to stressors, such as restraint and forced swimming, often produce offspring with impaired learning performance. However, it is unclear whether there are maternal effects on offspring learning when mothers are exposed to ecologically relevant stressors, such as predation risk. Here, we examined whether maternal predator-exposure affects adult offsprings' learning of a discrimination task in threespined sticklebacks (*Gasterosteus aculeatus*). Mothers were either repeatedly chased by a model predator (predator-exposed) or not (unexposed) while producing eggs. Performance of adult offspring from predator-exposed and unexposed mothers was assessed in a discrimination task that paired a particular coloured chamber with a food reward. Following training, all offspring learned the colour-association, but offspring of predator-exposed mothers located the food reward more slowly than offspring of unexposed mothers. This pattern was not driven by initial differences in exploratory behaviour. These results demonstrate that an ecologically relevant stressor (predation risk) can induce maternal effects on offspring learning, and perhaps behavioural plasticity more generally, that last into adulthood.

Keywords: behavioural plasticity; colour association; discrimination learning; maternal effects; maternal stress; transgenerational plasticity

1. INTRODUCTION

Learning is a form of phenotypic plasticity that allows organisms to adjust their behaviour to the environment and can affect a variety of important activities, such as food acquisition, predator avoidance and mate choice [1,2]. However, the ability to learn might not always be favoured owing to potential costs [3]. While there is evidence that learning performance might be heritable, it can also be influenced by the environment [1,2].

Cases in which an individual's learning performance is affected by its mother's environment are particularly intriguing [4]. For example, prenatal maternal stress can have negative impacts on offspring learning; an effect mediated by stress-induced changes in maternal hormones [5–8]. However, studies often use maternal stressors, such as restraint, foot shock or forced swimming, rather than stressors that might be relevant in nature, making the evolutionary interpretation difficult [5]. Other studies have shown that maternal exposure to ecologically relevant stressors, such as predation risk, affects many offspring behaviours [9–12], but the link between such stressors and offspring learning remains unexplored.

In this study, we used threespined stickleback (*Gasterosteus aculeatus*) to test whether female exposure to a model predator affected her adult offsprings' learning performance in a discrimination task. Previous studies in this system have shown that predator-exposure elevates cortisol in female stickleback [13] as well as their eggs [10], and affects offspring behaviour [10,11] and fitness [11].

2. METHODS

Groups of field-collected females (Putah Creek, CA, USA) were randomly assigned to either predator-exposed or unexposed treatment tanks. Predator-exposed females were chased for 30 s once a day by a Northern pike model (23 cm). Unexposed females were undisturbed. This protocol elevates cortisol in eggs of predator-exposed females [10] and affects offspring anti-predator and shoaling behaviour [10,11]. After at least 7 days in a treatment, when a female became gravid she was stripped of eggs that were artificially fertilized. Juveniles from at least five mothers of the same treatment were pooled and reared to adulthood. Thus, individuals used here are an unknown combination of full-siblings and unrelated individuals from at least 10 different families. Further details about maternal treatment and rearing methods are in McGhee *et al.* [11].

We used a discrimination learning protocol known to be effective in stickleback [14]. Blue and yellow 'L-shaped' walls were placed in the back corners of each test tank to create two chambers, each with a single entrance (see the electronic supplementary material). Because we were interested in assessing learning rather than colour *per se*, the blue chamber always contained the food reward (bloodworm-filled Petri dish) [14]. We alternated the tank side with the blue chamber between trials. Individuals had no prior feeding experience with either colour.

Individuals ($n = 9$ offspring of unexposed mothers, $n = 11$ offspring of predator-exposed mothers) were trained in eight discrimination learning trials: four trials on day 1 (09.00, 10.00, 13.00 and 14.00) and four trials on day 3 (09.00, 10.00, 13.00 and 14.00). After training, they were tested again (day 5: 09.00).

After 24 h of food restriction, individuals were herded into water-filled cups and transferred to individual test tanks where they remained for the entire experiment. Before each trial, an opaque holding cylinder was lowered over individuals and moved into position. Individuals acclimated in this holding cylinder for 1 min while chambers and food reward were added. They were then released and watched for 10 min. If they located the food reward during the trial, they could feed for 30 s. In the first trial, if an individual failed to find the food reward, it was led to it using a bloodworm-filled pipette. In subsequent trials, if an individual failed to find the food reward, it was not led to it, received no food and was given a score of 600 s. Between trials, individuals could swim freely in their test tanks but the chambers and reward were removed and individuals were allowed to see neighbouring fish. Fish were not fed outside trials so the only available food was the food reward. After the final trial, we measured standard length. See the electronic supplementary material for additional methods.

Offspring from the two maternal treatments might differ in their tendency to explore the novel tank and chambers resulting in differences in their likelihood of initially finding the food. To examine this, we compared maternal treatments 'before training' (day 1) in how quickly individuals began moving after release from their holding cylinder, and how quickly they entered their first chamber regardless of whether it was the rewarded chamber, using ANCOVAs with standard length as a covariate.

Table 1. Behaviours (mean ± s.e.) of the offspring from the maternal treatments.

	offspring of predator-exposed mothers (s)	offspring of unexposed mothers (s)
initial exploratory behaviour (day 1: 09.00):		
latency to first begin moving	49 ± 30	56 ± 20
latency to enter either chamber for the first time	330 ± 70	326 ± 78
learning the colour association:		
day 1 (09.00): latency to find food reward	426 ± 65	427 ± 61
day 3 (09.00): latency to find food reward	533 ± 48	304 ± 74
day 5 (09.00): latency to find food reward	337 ± 61	158 ± 68

Table 2. Results of statistical analyses.

	estimate ± s.e.	d.f.	*F*-value	*p*-value
latency to first begin moving (day 1: 09.00):				
maternal treatment	0.321 ± 0.471	1,18	0.46	0.506
standard length	−0.049 ± 0.055	1,18	0.79	0.386
latency to enter either chamber for the first time (day 1: 09.00):				
maternal treatment	0.072 ± 0.406	1,18	0.03	0.862
standard length	−0.067 ± 0.047	1,18	2.04	0.172
latency to find food reward:				
maternal treatment	−0.105 ± 0.361	1,16	2.95	0.105
trial	0.245 ± 0.223	1,17	21.98	0.0002
maternal treatment × trial	1.121 ± 0.344	1,17	10.64	0.005
standard length	−0.058 ± 0.037	1,16	2.48	0.135

To examine whether offspring from the two maternal treatments differed in their ability to learn the colour-reward association, we compared them in how quickly individuals found the food (i.e. pecked at food or food dish) using a repeated measures ANCOVA with individual as the subject and standard length as a covariate. Because we were interested in how training affected performance, we compared maternal treatments 'before training' (day 1: 09.00) and 'after training' (day 5: 09.00). One individual from the unexposed maternal treatment never found the food and was excluded. Analyses were conducted with SAS, v. 9.2. All data were natural log-transformed, and we validated model assumptions by examining residuals. Non-significant interactions with the covariate were removed. All data have been deposited in Dryad (doi:10.5061/dryad.12j03).

3. RESULTS

Prior to training, offspring of predator-exposed and unexposed mothers did not differ in their exploratory behaviour and they took similar amounts of time to begin moving and to enter a novel chamber (tables 1 and 2). In addition, they initially took similar amounts of time to find the food reward (table 1). Offspring from both maternal treatments improved in finding the food following training, consistent with learning (figure 1). This improvement was much greater in offspring of unexposed mothers compared with offspring of predator-exposed mothers, resulting in a strong treatment × trial interaction (tables 1 and 2). Following training, offspring of unexposed mothers were 63 per cent faster at finding the food reward compared with before training, while offspring of predator-exposed mothers were only 21 per cent faster. This differential improvement in finding the food was evident midway through training (table 1).
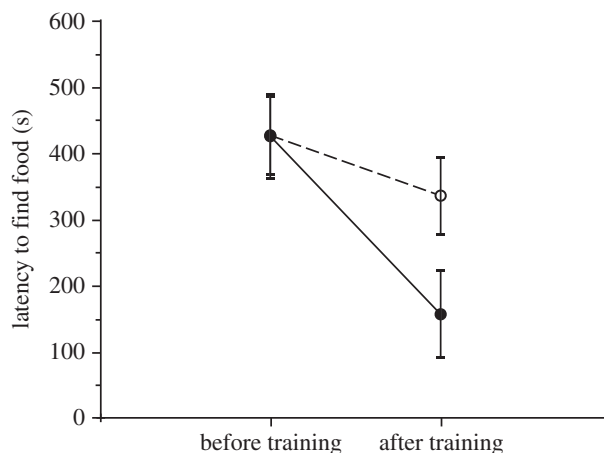

Figure 1. Offspring of predator-exposed mothers (dashed line with open circles) showed less improvement in finding the food reward following training (mean ± s.e.) compared with offspring of unexposed mothers (solid line with filled circles; 'before training': 09.00 trial on day 1, 'after training': 09.00 trial on day 5).

Not every individual found the food in each trial. Before training, 63 per cent of offspring from unexposed mothers (five of eight) and 55 per cent of offspring from predator-exposed mothers (six of 11) found the food reward. The number of individuals finding the food reward increased with training. After training, 88 per cent of offspring from unexposed mothers (seven of eight) and 73 per cent of offspring from

predator-exposed mothers (eight of 11) found the food reward. Removal of individuals that did not locate the food from the analysis did not change the results (latency to find food: maternal treatment: $F_{1,13.5} = 1.72$, $p = 0.212$; trial: $F_{1,10.7} = 13.05$, $p = 0.004$; maternal treatment × trial: $F_{1,10.6} = 10.66$, $p = 0.008$).

4. DISCUSSION

In this study, we found that offspring of predator-exposed mothers showed less improvement in locating a food reward following training compared with off-spring of unexposed mothers. This pattern was not driven by initial differences in how quickly individuals began to explore the tank or their tendency to enter a novel chamber for the first time. These results demonstrate that an ecologically relevant stressor (predation risk) can induce a maternal effect on offspring learning that is surprisingly long-lasting [4], having an effect well into adulthood. Interestingly, our results are consistent with the finding that stickleback collected from low-predation rivers solve spatial tasks faster than those from high-predation rivers [15] suggesting that the pattern in field-collected individuals could be, at least partially, owing to maternal effects.

Maternal predator-exposure was linked to learning deficits in adult offspring, consistent with other studies showing impaired learning in offspring of stressed mothers [5–7]. In addition, maternal predator-exposure in threespined sticklebacks has been linked to negative effects on offspring anti-predator behaviour and survival with a live predator [11], as well as shoaling differences [10]. Together, these results suggest that maternal stress might affect the overall ability of offspring to modify their behaviour in response to the environment resulting in consequences for behavioural plasticity across a number of contexts. This is consistent with recent studies finding that maternal stress can affect offspring emotional and social reactivity [16,17]. Whether these negative effects are balanced by positive effects on other traits is unknown. Measuring a variety of offspring behaviours under diverse conditions could provide insight into how maternal predator-exposure affects behavioural plasticity in general and the overall fitness of offspring.

The effect of maternal stress on offspring learning might be mediated by glucocorticoid stress hormones. Higher levels of cortisol have been implicated in learning deficits in other species [5,7,8]. Although we did not manipulate cortisol directly, studies in sticklebacks have found that females exposed to a predation risk have higher levels of circulating cortisol [13], as do their eggs [10]. Thus, our results fit well with the hypothesized mechanism that predator-exposed females are exposing their offspring prenatally to elevated levels of cortisol that affect offspring learning.

Although the maternal effect on offspring learning demonstrated here might seem maladaptive, the benefits (and costs) of learning are environment- or context-dependent [1–3]. Importantly, we found that offspring of both maternal treatments successfully learned the colour association but differed in their speed at obtaining the food. The maternal effect seen here could well be adaptive if predator-exposed mothers are 'preparing' their offspring for a high-predation environment where the induced costs of learning and memory outweigh the benefits of having enhanced learning performance [3], or where increased vigilance and caution is favoured at the expense of obtaining a learned food source quickly [15]. Examining how learning performance and behaviour in a variety of learning paradigms affect overall fitness would provide insight into the adaptive nature of this maternal effect.

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