Social transmission of fear is disrupted by pharmaceutical pollution

Jack L. Manera [1,*], Eleanor R. Moore [1], Mirjam Amcoff [2], Bob B.M. Wong [1,†], Jake M. Martin [1,3,4,5,†], Niclas Kolm [6,†], Michael G. Bertram [1,4,6,†]

[1] *School of Biological Sciences, Monash University, Melbourne 3800, Australia*

[2] *Department of Aquatic Resources, Swedish University of Agricultural Sciences, Uppsala 750 07, Sweden*

[3] *School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Victoria 3216, Australia*

[4] *Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, Umeå 901 83, Sweden*

[5] *School of Agriculture, Biomedicine and Environment, La Trobe University, Melbourne, Victoria 3086, Australia*

[6] *Department of Zoology, Stockholm University, Stockholm 106 91, Sweden*

* Corresponding author: J.L. Manera, Jack.Manera@monash.edu

† Co-senior authors.

Jack L. Manera, ORCID: 0009-0003-7947-6473

Eleanor R. Moore, ORCID: 0009-0002-5204-1333

Mirjam Amcoff, ORCID: 0000-0002-1439-4691

Bob B.M. Wong, ORCID: 0000-0001-9352-6500

Jake M. Martin, ORCID: 0000-0001-9544-9094

Niclas Kolm, ORCID: 0000-0001-5791-336X

Michael G. Bertram, ORCID: 0000-0001-5320-8444

Abstract

Social information transfer enables rapid responses to threats and underpins collective predator avoidance across taxa. Yet whether the growing threat of environmental pollutants, such as pharmaceuticals, disrupt this process remains unknown. Here, we show that the widespread antidepressant pollutant amitriptyline disrupts fear contagion—the automatic matching of an individual's behavioural state to that of a frightened conspecific—in the guppy (*Poecilia reticulata*). Amitriptyline exposure impaired the translation of socially acquired threat information into behavioural responses and weakened behavioural coupling with distressed conspecifics, while responses to directly detected threats remained unaffected. These findings provide empirical evidence that pollution can degrade the dynamics of fear contagion—a fundamental mechanism underpinning rapid, socially mediated responses to predation risk. By eroding the integrity of social information networks, psychoactive contaminants may compromise coordinated antipredator behaviour and reshape predator–prey dynamics in polluted ecosystems, revealing a previously unrecognised dimension of ecotoxicological risk.

Keywords: amitriptyline, antidepressant, antipredator, collective behaviour, ecotoxicology, emotional contagion, fear contagion, information transfer, *Poecilia reticulata*, psychoactive

Introduction

Information is a fundamental resource that animals use to guide decision making in variable environments (1). It can be acquired directly through personal sampling of the environment or indirectly through social interactions with conspecifics (2, 3). The extent to which individuals rely on personal versus social information is shaped by the costs of acquiring the information, as described by the 'costly information hypothesis' (4). These costs can include energy expenditure, time and opportunity losses, and increased exposure to predation risk (5). When such costs are high, individuals may preferentially rely on social information as a lower-cost alternative, allowing them to make adaptive decisions without engaging in potentially inefficient and risky trial-and-error (6); but see (7).

Information concerning predation risk is among the most consequential information an animal can acquire. This is because predation risk imposes immediate and potentially lethal consequences. Social information is particularly valuable in this context as it allows threat information to be acquired indirectly, removing the need for each individual to independently verify the presence or absence of risk (6). One of the most ancient and widespread mechanisms underpinning the social transmission of threat information is emotional contagion (8, 9). Emotional contagion—also referred to as 'fear contagion' when pertaining to threat information—is the automatic matching of an observer's behavioural or physiological state to that of a demonstrator (10). Fear contagion emerges through perception–action coupling, whereby the perception of fear-related behaviour in others activates corresponding internal states in the observer, leading to congruent behavioural responses (11). Because observers in fear contagion paradigms do not directly detect the threat themselves, all subsequent updating of threat estimates must also rely on social information (12, 13)—specifically, the accumulation of socially transmitted safety cues as demonstrators resume normal activity (14, 15). In this way, fear contagion initiates a broader antipredator response, while the persistence and resolution of that response are dynamic and depend on continued sampling and integration of social information over time.

Emotional contagion is considered the most fundamental form of empathy (10). It has been documented across a remarkably wide range of taxa, including both vertebrates (reviewed in; (8) and invertebrates (16), and an increasing body of work points to highly conserved neural and neurochemical substrates underlying emotional contagion across species (17–19). Accordingly, experimental manipulations altering these neurochemical systems have been

shown to produce substantial changes in fear contagion responses (17). It then follows that disturbances capable of disrupting these neurochemical pathways may also alter how animals perceive, process, and respond to socially transmitted threat information. Yet, whether anthropogenic disturbances compromise fear contagion—and thus the reliability of socially transmitted threat information—remains untested.

Among anthropogenic disturbances, psychoactive pharmaceutical pollutants represent a mechanistically plausible threat to socially transmitted fear responses. This is because these compounds are specifically designed to modify neurochemical pathways (20), are routinely detected in aquatic ecosystems worldwide (21), and have demonstrable effects on behaviour in non-target organisms (22–25). Amitriptyline, a widely prescribed tricyclic antidepressant, exemplifies the hazards posed by pharmaceutical pollutants. By acting on dopaminergic, serotonergic, and adrenergic pathways, it produces broad neurochemical shifts (26) and has documented neuroactive effects in exposed wildlife (27, 28). Moreover, it is frequently detected in freshwater systems globally (21) and has been shown to bioaccumulate in the neural tissue of fish (29). Yet, whether psychoactive contaminants such as amitriptyline impair fear contagion remains unknown, despite the potential for this disruption to destabilise group-level antipredator dynamics (30) and population-level outcomes (31).

Here, we tested whether exposure to environmentally realistic concentrations of the widespread psychoactive pharmaceutical pollutant amitriptyline (21, 32) affects fear contagion in freshwater fish. We focused on adult female guppies (*Poecilia reticulata*) as they exhibit pronounced shoaling behaviour and a strong reliance on social interactions (33), making them well-suited for testing socially transmitted threat responses. Observer fish (N = 162) were housed for 10 d in one of three treatment conditions: an unexposed control, a low concentration amitriptyline treatment (31 ng L^{-1}), or a high concentration amitriptyline treatment (175 ng L^{-1}). Following exposure, each observer was transferred to a paired-tank assay in which a single exposed observer was housed adjacent to a shoal of three unexposed demonstrator fish (N = 162; Figure 1A). Observer fish received threat information via one of two modes. In the social-information condition, chemical alarm cue (also known as ‘Schreckstoff’; 34) was pipetted only into the demonstrators’ tank, eliciting antipredator behaviour in the demonstrator shoal; observers therefore received threat information solely through socially transmitted visual cues. In the combined-information condition, chemical alarm cue was added simultaneously to both the demonstrators’ and observer’s tanks, so observers received direct personal confirmation of

the risk together with the socially transmitted threat cues. We tracked the behaviour of observers and demonstrators throughout the trial and quantified the observers' fear responses and recovery, as well as their behavioural coupling with their demonstrator shoals.

We predicted that combined threat information would elicit stronger antipredator responses in the observer fish than social information alone, as personal threat detection confirms the social signal, creating convergent evidence that amplifies threat assessment (see social facilitation versus social contagion in 35). We further predicted that amitriptyline-exposed observers would exhibit altered recovery dynamics and weakened behavioural coupling with the demonstrator shoals compared to unexposed observers. This is consistent with the known effects of antidepressant pollutants reducing anxiety-related behaviours (36, 37). Critically, we predicted that this disruption in fear response would be disproportionately greater in the social-information condition, given amitriptyline's action on the neurochemical pathways mediating fear contagion (17, 38). This study is the first empirical assessment of whether and how chemical pollution can disrupt fear contagion, with broad implications for social coordination and predator–prey dynamics in contaminated environments.

Results

Guppies exhibit robust fear contagion and personal confirmation amplifies this response

Observer guppies exhibited strong fear reactions to threat information across both information conditions, characterised by prolonged immobility following cue delivery and a gradual return to pre-stimulus activity levels (Figure 1B–D). We quantified the recovery dynamics using two time-to-event metrics: latency to resume movement (min), which is the time elapsed from cue delivery until the observer first recommenced movement (39), and time to recover (min), defined as the time required for both swimming velocity and proportion of time moving to return to, and remain at, pre-stimulus baseline levels—identified using a composite similarity score standardised to each individual's own baseline behaviour (see Materials and Methods for full definitions).

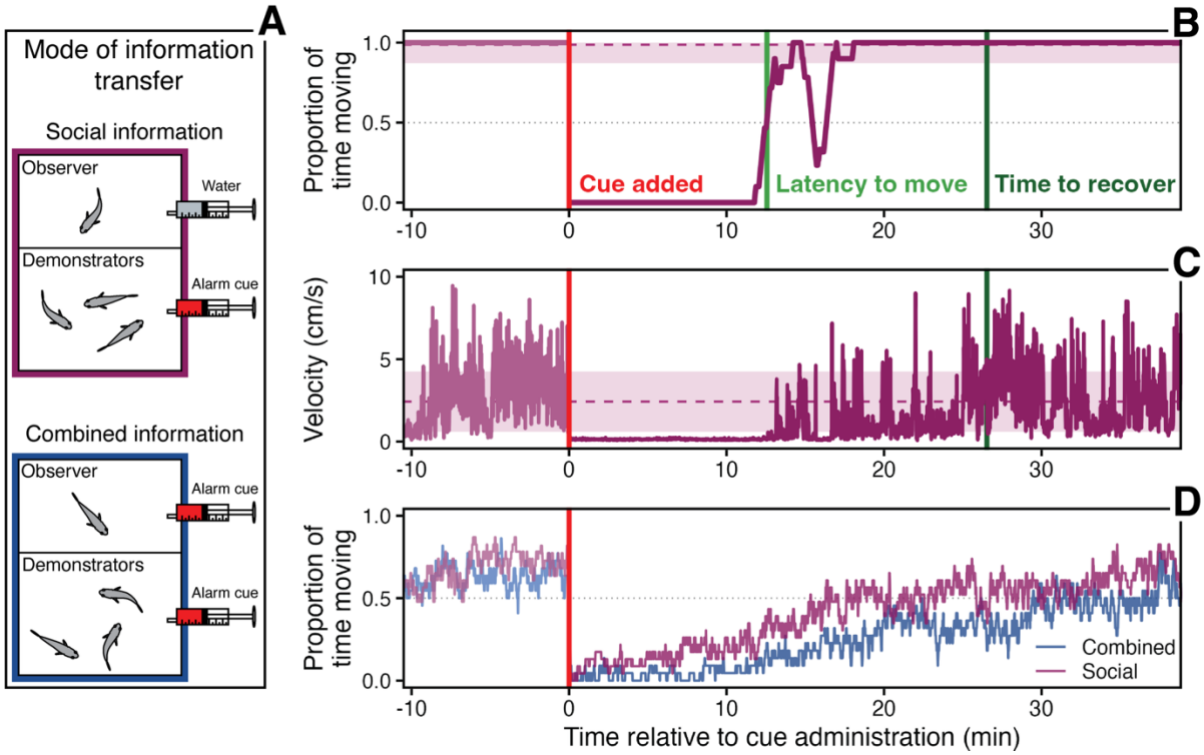

Figure 1. Temporal dynamics of fear contagion behaviour and detection of a threat. (A) Experimental design diagram illustrating the two modes of information acquisition. In the social condition, observer fish received visual cues from demonstrator fish subjected to a chemical alarm cue, while in the combined condition, observers directly received the alarm cue themselves together with the social visual cues. (B, C) Representative behavioural time series for a single unexposed observer fish receiving socially transmitted information about a threat. The red vertical line marks the time of alarm cue administration to the demonstrator tank (time = 0 min). Panel (B) shows the proportion of time spent moving, calculated using a 60-s sliding window, and panel (C) shows swimming velocity (cm s^{-1}). The shaded regions represent ±1 SD around the pre-cue baseline mean (horizontal dashed line) for each behavioural metric. Following cue administration, the observer exhibits a marked reduction in activity consistent with a socially transmitted fear response. The light green vertical line indicates the latency to move, defined as the time at which the fish resumes movement (> 50% of time spent moving). The dark green vertical line marks time to full recovery, defined as the first changepoint at which a composite similarity metric—based on velocity and proportion of time spent moving relative to baseline—indicates that behaviour has returned to the baseline for the remainder of the trial. (D) Population-level behavioural responses. Lines show the mean proportion of time spent moving for fish receiving socially transmitted information (purple; n = 71) and combined information (blue; n = 62). The full trial spanned 20 min before and 90 min after cue administration; panels (B–D) show a subset of this period positioned around cue administration.

156 The mode of threat information transfer substantially influenced recovery dynamics. In the
157 unexposed group, fish that received the combined information resumed movement after 27.4
158 min (95% CrI: 17.8, 43.5) and recovered to baseline after 50.8 min (95% CrI: 22.7, 106.3),
159 whereas socially informed fish resumed movement faster after only 12.5 min (95% CrI: 8.0,
160 19.8) and recovered after 32.8 min (95% CrI: 14.6, 69.3; Figure 2A, C; Table S1). This means
161 that fish that received combined information took more than twice as long to resume movement
162 than socially informed fish (Δ = 12.6 min; 95% CrI: 5.5, 22.2; probability of direction *pd* =
163 1.00; Figure 2C; Table S2) and 55% longer to recover to baseline, (Δ = 20.8 min; 95% CrI:
164 4.0, 54.0; *pd* = 0.99; Figure 2A; Table S2). Together, these results demonstrate robust fear
165 contagion in guppies and reveal that personal corroboration of social threat information
166 substantially prolongs their recovery. This pattern suggests that recovery depends on the
167 information available for updating the perceived level of risk: animals integrate personal and
168 social cues when assessing predation risk, but the weight assigned to each cue depends on its
169 reliability and availability (5). Observers with combined information had both personal and
170 social evidence of danger; thus, their recovery is a reflection of threat estimation integrating
171 both social safety cues as well as personal threat extinction. Socially informed observers,
172 however, had only social threat cues to inform their threat estimate. Therefore, in the absence
173 of personal confirmation of the threat, confidence in socially acquired threat information may
174 decay over time, leading to faster recovery.

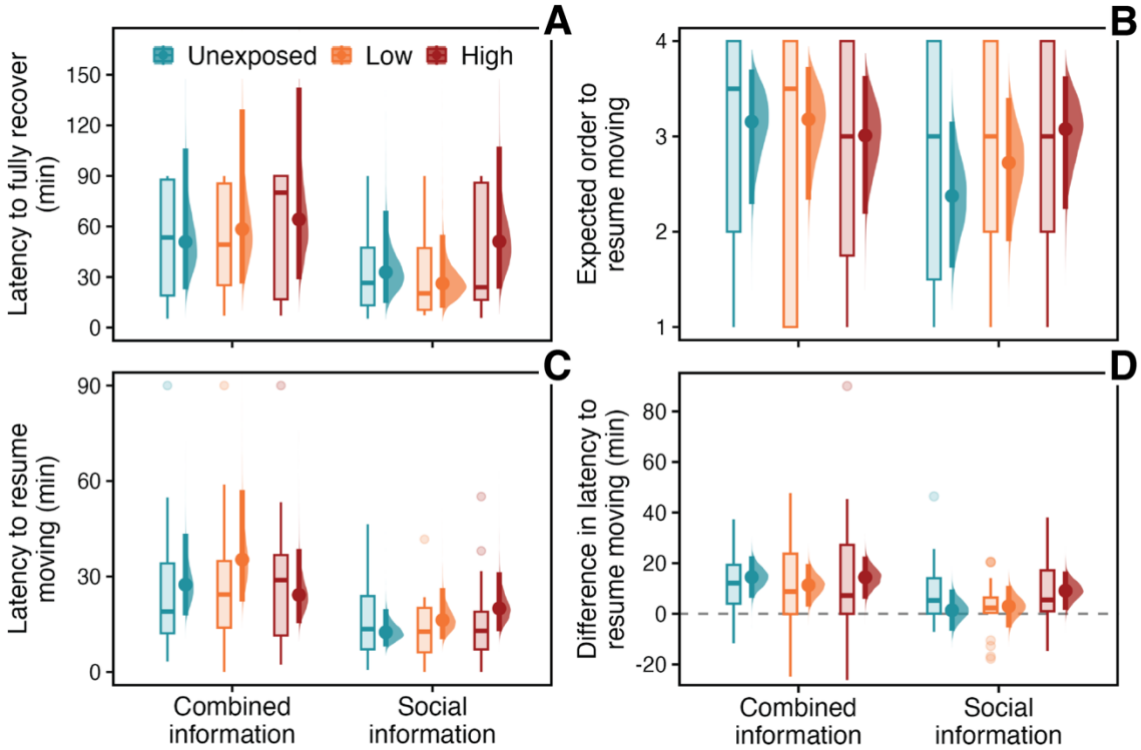

Figure 2. Effects of amitriptyline exposure on behavioural responses to socially and personally acquired threat information. Behavioural response metrics are shown for fish receiving socially transmitted threat information or a combination of social and personal information, under unexposed (teal; 0 ng L^{-1}; $n_{combined} = 22$, $n_{social} = 23$), low (orange; 31 ng L^{-1}; $n_{combined} = 20$, $n_{social} = 23$), and high (red; 175 ng L^{-1}; $n_{combined} = 20$, $n_{social} = 25$) amitriptyline exposure treatments. Boxplots display raw data distributions, with central lines indicating medians, boxes representing interquartile ranges, and whiskers extending to 1.5× the interquartile range; points and error bars show model-estimated means and 95% credible intervals, with posterior distributions shown as density plots. (A) Latency to fully recover, defined as the time for behaviour to return to and remain at baseline activity levels. (B) Relative order of resuming movement, where lower values indicate earlier recovery relative to other individuals. (C) Latency to resume movement, defined as the time at which the fish first resumes movement. (D) Temporal lag between the first demonstrator resuming movement and the observer fish resuming movement; negative values indicate that the observer resumed movement before any demonstrator. Across all metrics, responses differed between information sources and treatment groups, demonstrating that amitriptyline exposure impaired fear contagion—the behavioural response to socially transmitted threat information—while leaving general fear response to direct detection of a threat intact.

Amitriptyline exposure disrupts the social transmission of fear

Amitriptyline exposure substantially altered fear response dynamics in socially informed fish, while fish receiving the combined information were unaffected. In the social-information

condition, unexposed fish took 12.5 min (95% CrI: 8.0, 19.8) to resume moving and 32.8 min (95% CrI: 14.6, 69.3) to recover to their baseline behaviour, compared to 20.0 min (95% CrI: 12.8, 31.4) and 51.1 min (95% CrI: 23.1, 107.4) in high-exposed fish—representing a 60% and a 56% increase respectively (high – unexposed: Δ latency to move = 7.0 min, 95% CrI: −0.7, 16.5, *pd* = 0.96; Δ recovery time = 17.2 min, 95% CrI: −6.1, 57.1, *pd* = 0.93; Figure 2A, C; Table S3). Low-exposed fish showed recovery dynamics broadly comparable to unexposed fish, indicating that detectable disruption was primarily evident at the higher exposure concentration (low – unexposed: Δ latency to move = 3.5 min, 95% CrI: −3.4, 12.0, *pd* = 0.85; Δ recovery time = −6.0 min, 95% CrI: −30.5, 10.9, *pd* = 0.79; Figure 2A, C; Table S3). By contrast, fish with access to the combined information were unaffected by amitriptyline at either concentration (all *pd* < 0.90 for latency to resume movement and recovery to baseline; Figure 2A, C; Table S3), demonstrating that amitriptyline selectively impaired the behavioural response to socially transmitted, but not personally detected, threat information.

Amitriptyline alters observer–demonstrator behavioural coupling

The resumption of movement following a fear response is a process that requires continuously monitoring demonstrator behaviour, updating threat estimates, and ultimately judging when it is safe to recommence activity (40). This decision unfolds in the context of a demonstrator shoal recovering at its own pace. As each demonstrator moves in turn, the cumulative social signal of safety grows stronger (12, 41). The timing with which an observer resumes movement, therefore, reflects the weight of social evidence an individual requires before judging threat levels to be low enough to warrant resumption of movement (13, 15). To quantify this, we recorded two complementary indices of how closely observer recovery was coupled to demonstrator recovery: (1) the rank order in which observers resumed movement within the group, and (2) the elapsed time between the first demonstrator to resume movement and the observer to resume movement. Each group comprised four fish, one observer and three demonstrators, such that resumption order was ranked 1 (first fish to move) through 4 (last fish to move).

Resumption order differed systematically by information condition. Under unexposed conditions, observers with combined information resumed movement relatively late in the sequence, moving approximately third out of four fish (mean estimate = 3.15, 95% CrI: 2.29, 3.70; Figure 2B; Table S1). Socially informed observers resumed movement earlier in the

sequence, ranking closer to second (mean estimate = 2.37, 95% CrI: 1.62, 3.15; Figure 2B; Table S1). Amitriptyline exposure altered this pattern in socially informed fish, where high-dose observers resumed movement later in the sequence relative to unexposed fish (high – unexposed: Δ = 0.66, 95% CrI: −0.09, 1.41, *pd* = 0.96; Figure 2B; Table S3). Low-dose observers, however, demonstrated an intermediate shift that did not meaningfully differ from either unexposed or high-dose fish (low − unexposed: Δ = 0.33, 95% CrI: −0.47, 1.10, *pd* = 0.79; high − low: Δ = 0.33, 95% CrI: −0.44, 1.09, *pd* = 0.81; Figure 2B; Table S3).

To complement the rank-order data, we also measured the absolute temporal gap between the observer and the leading demonstrator. Here, the 'leading demonstrator' refers to the first of the three demonstrators to resume movement and thus represents the earliest available social cue that conditions may be safe. These two measures—resumption order and observer–demonstrator latency difference—are intrinsically linked rather than independent, because observers that rank later in the sequence will also resume movement later relative to the first demonstrator, all else held equal. Unsurprisingly, latency to resume movement followed the same pattern as resumption order, varying by both information condition and drug exposure. On average, socially informed observers resumed movement substantially sooner after the leading demonstrator than observers with the combined information (social – combined: Δ = −8.84 min, 95% CrI: −15.09, −2.36, *pd* = 1.00; Figure 2D; Table S2), consistent with the tighter behavioural coupling expected when social information alone drives the fear response and thus also the recovery. Among socially informed fish, amitriptyline exposure widened this temporal gap, whereby high-dose observers resumed movement substantially later relative to the leading demonstrator compared to unexposed fish (high − unexposed: Δ = 7.74 min, 95% CrI: −2.50, 18.09, *pd* = 0.93; Figure 2D; Table S3). Taken together, the resumption order and latency data suggest that the socially informed fish exposed to amitriptyline required substantially more accumulated social evidence of safety before resuming movement. Neither resumption order nor latency difference varied meaningfully with amitriptyline dose in the combined-information condition, mirroring the absence of drug effects on the recovery dynamics of directly informed fish reported above and confirming that amitriptyline selectively disrupts the processing of socially transmitted, rather than personally detected, information.

Environmentally relevant amitriptyline exposure

To verify amitriptyline concentrations over the course of the experiment, water samples were collected from a subset of 48 tanks and analysed using liquid chromatography–tandem mass spectrometry (LC–MS/MS; see Materials and Methods for full details). The estimated marginal mean concentration in the low treatment group was 31 ng L^{-1} (95% CrI: 17.57, 61.19, n = 18) and 175 ng L^{-1} (95% CrI: 101.31, 351.01, n = 18) in the high treatment group. Amitriptyline was not detected in any of the control samples (below the detection limit of 5 ng L^{-1}; n = 12), confirming the absence of cross-contamination. Although the high treatment group fell below the target nominal concentration—likely attributable to biodegradation and sorption (42)—the measured concentrations demonstrated clear separation between groups and represent environmentally relevant concentrations. Both low and high treatment concentrations fell within the range reported in environmental monitoring studies (up to 102 ng L^{-1} in surface waters and 355 ng L^{-1} in direct effluent outflow; 21, 43), with the low treatment group approaching mean environmental concentrations (26 ng L^{-1}, N = 418; 44).

Discussion

Here, we show for the first time that environmentally realistic exposure to a pharmaceutical pollutant can disrupt the way socially acquired information is translated into behavioural decisions. As predicted, fish exhibited robust antipredator responses to socially produced threat information and these responses were strongly amplified when social cues were corroborated by direct personal detection of the threat. Critically, when exposed to the high concentration of amitriptyline, fish relying on social cues were slower to recover and showed altered behavioural coupling with demonstrators compared to unexposed fish. In contrast, amitriptyline exposure had no meaningful effect when observers received threat information both directly and socially. Taken together, these findings reveal a previously unrecognised pathway by which psychoactive contaminants can degrade the reliability of social information use, with widespread potential consequences for coordinated antipredator behaviour in contaminated wildlife populations (30, 31).

Observer fish that received both personal and social threat information exhibited stronger and more prolonged antipredator responses than fish receiving social information alone, taking more than twice as long to resume movement and ~55% longer to recover to baseline behaviour. This pattern is consistent with information-based models of antipredator decision-making, which posit that individuals integrate multiple cues when assessing danger and scale

their responses to the combined evidence (2). When personal detection corroborates social information, the two sources of evidence reinforce one another, producing a stronger and more sustained response than either would elicit in isolation (45)—a pattern described by Oliveira and Faustino (35) as social facilitation of antipredator behaviour. This difference likely reflects both the greater quantity of information available to the individuals that received combined information and the higher reliability of chemical alarm cues. Because chemical cues are released only upon conspecific injury, they provide an unambiguous indicator of risk, whereas behavioural cues are noisier and require interpretation and are thus less reliable (46, 47). An alternative explanation for this difference between the information conditions is that chemical alarm cues persist longer than socially produced behavioural cues, prolonging the threat signals for the observers in the combined-information condition. However, behavioural responses to alarm cues fade rapidly through sensory habituation even when chemical cues remain present, suggesting that perceived risk decays faster than cue persistence alone would predict (48). Moreover, demonstrator recovery (the social signal itself) should track declining perceived risk rather than absolute cue concentration, so socially transmitted signals are likely to operate on a similar timescale to the effective informational value of the chemical cue. Cue persistence alone, therefore, seems unlikely to explain the different recovery dynamics between the information conditions.

A key finding from this study is the selective disruption of responses to socially transmitted threat information by amitriptyline. Observers exposed to high concentrations of amitriptyline and relying on social cues alone took longer to resume movement, recovered more slowly to baseline behaviour, and showed weaker behavioural coupling with demonstrators than unexposed fish. By contrast, amitriptyline exposure had no meaningful effect when observers could directly detect the threat themselves. This dissociation suggests, under the conditions tested here, that amitriptyline did not generally alter fear or anxiety states. Instead, it appears to have impaired the processing and translation of socially acquired threat information into action. If amitriptyline increased or dampened fear responses in a general sense, treatment effects would be expected across both social- and combined-information contexts. Interpreting the effects in the social-information condition requires recognising that antipredator behaviour unfolds as a dynamic ongoing process, rather than a single event (49). The behavioural response to threats can be broadly divided into two temporally distinct phases: an initial rapid reaction to a perceived threat, followed by a more evaluative phase in which individuals continuously reassess risk and determine when it is safe to resume other activities (50, 51). The

initial onset of antipredator behaviour in fear contagion is largely automatic and reflexive, arising from perception–action coupling (10). In our study, this initial reaction occurred extremely quickly following alarm cue delivery to the demonstrators (interquartile range of observer reaction time < 1 s). Although a supplementary analysis revealed no evidence for treatment effects on reaction time (Supplementary Information S1), this narrow temporal window—combined with uncontrolled variability in cue diffusion and observer attention—leaves little resolution to detect treatment differences, and we therefore refrain from inferences regarding this phase.

Amitriptyline's effects instead clearly emerged during the later evaluative recovery phase. During this phase, observers continually update their threat estimates by monitoring demonstrators' behaviour for accumulating social safety cues (15). Under unexposed conditions, socially informed observers typically resumed movement after only a single demonstrator began moving, indicating that a relatively small amount of social safety information was sufficient to reduce perceived risk below the threshold required to maintain freezing behaviour. In contrast, amitriptyline-exposed observers took longer to resume movement and did so later in the recovery sequence, often only after multiple demonstrators had already become active. This pattern indicates a marked shift in how social information was translated into action. We contend that amitriptyline reduced the precision with which social cues were perceived or integrated, so that each cue carried less evidential weight and a larger accumulation of cues was required before observers could infer that it was safe to resume activity. Alternatively, exposed fish may have defaulted to simpler, more robust social heuristics, such as 'copy-the-majority' or 'copy-when-uncertain' strategies (52). These group copying rules are known to be favoured when personal or social information is unreliable, thus serving as adaptive fallbacks under uncertainty (53, 54). Both interpretations are consistent with the potential cognitive disruptions that have been reported when fish are exposed to amitriptyline (28) and, crucially, both point to the same outcome: amitriptyline exposure weakened the functional coupling between observer and demonstrator behaviour by degrading the observer's ability to translate early social cues into confident action. To extend the generality of these findings, future work could examine whether similar effects occur in males, as only females were tested here, particularly given the documented sex differences in social information use (55) and the sex-specific cognitive effects of amitriptyline (28).

Social transmission of fear recruits evolutionarily conserved neurochemical pathways (17, 19), with oxytocin signalling playing a critical role in fear contagion across taxa (17, 18). One of

amitriptyline's primary pharmacological actions is serotonergic reuptake inhibition, which has been shown to modulate social decision-making (56), and is linked to altered oxytocin levels across brain regions (38), providing a plausible neurochemical mechanism for the disruption we observed. Notably, amitriptyline bioaccumulates preferentially in the telencephalon and hindbrain of fish (57), which is particularly relevant given that the integration of socially transmitted threat information in guppies is associated with increased neural activity in the telencephalon (47). Together, these observations point to a parsimonious mechanistic pathway whereby amitriptyline accumulates in the very brain regions that mediate fear contagion and modulate the oxytocinergic and monoaminergic signalling on which this behaviour depends.

When interpreting the magnitude of the effects reported here, it is important to recognise the role of social feedback loops. This is because the behaviour of observer and demonstrator fish could influence one another bidirectionally. These social feedback loops almost certainly shaped the responses we observed. Social buffering—the dampening of fear by cues from relaxed conspecifics—may have attenuated demonstrator fear in the social information treatment, thereby weakening the social threat cue transmitted to observers. Conversely, social facilitation—the amplification of fear when social and personal threat cues converge—likely exacerbated demonstrator responses in the combined-information condition (35). These same feedback loops may have also amplified the amitriptyline exposure effects. A unidirectional design could, in principle, isolate these contributions; however, we argue that preserving bidirectional feedback enhances, rather than compromises, the ecological relevance of our findings, because wild shoals are inherently reciprocal social environments in which every individual's behaviour feeds back into the collective response.

The ecological implications of these findings extend beyond the individual fish tested here. Social information underpins collective antipredator behaviour by enabling rapid transmission of threat cues through groups (58), thereby enhancing coordinated evasion and reducing predation risk (59). Importantly, socially transmitted information also allows defensive behaviours to be flexibly regulated, such that energetically costly antipredator responses can be relaxed once group members signal that the immediate risk has passed (40). By selectively impairing how observers translate social information into action, amitriptyline exposure is likely to degrade both ends of this process. Exposed individuals may fail to adopt protective behaviours when risk is high, and may also fail to resume foraging, mating, and other fitness-relevant activities once the threat has subsided. Both outcomes carry clear fitness costs, elevated predation mortality on the one hand, and lost opportunities for energy acquisition and

reproduction on the other (50). These effects occurred at concentrations already measured in surface waters receiving wastewater effluent (21, 43), indicating that populations inhabiting contaminated catchments may already experience erosion of social information transfer, with consequences scaling from the individual to the group (60). These disruptions to the flow of social information represent a subtle but potentially pervasive pathway by which pharmaceutical pollution could reshape predator–prey dynamics and, ultimately, community structure in contaminated environments (60).

In summary, we provide the first evidence that exposure to a widespread pharmaceutical pollutant at environmentally realistic concentrations can disrupt the dynamics of fear contagion, selectively impairing the uptake and integration of socially transmitted information while leaving responses to directly detected threats intact. Because fear contagion underpins the rapid transmission of threat information across a wide range of taxa, its disruption has clear potential to compromise coordinated antipredator behaviour in contaminated environments. More broadly, our findings show that pharmaceutical pollutants can erode not only individual behaviour but also the integrity of the social information networks on which many species depend—highlighting a previously overlooked dimension of ecotoxicological risk.

Materials and Methods

Experimental design

A total of 348 adult female guppies (*P. reticulata*) were used in this study. These fish were sourced from a laboratory population established with wild-caught fish from high-predation sites in the Quare River, Trinidad and Tobago (61). All exposure and experimental procedures were conducted under Stockholm University animal ethics approvals (Project numbers: 17362-2019 and 12211-2021 with amendment 5982-2024). Of these, 162 individuals functioned as observer fish, while an additional 162 individuals functioned as demonstrator fish. Observer fish were assigned to one of three exposure groups: unexposed (0 ng L^{-1} amitriptyline nominal, n = 54), low amitriptyline (30 ng L^{-1} nominal, n = 54), or high amitriptyline (300 ng L^{-1} nominal, n = 54). Within each exposure group, observer fish were further subdivided such that 27 individuals received only socially transmitted information (social-information condition), whilst the other 27 individuals received both social information and personal information in combination (combined-information condition). Demonstrator fish were housed in groups of

three individuals (housing tank dimensions: 24 × 14 × 13 cm; 2.6 L), with each group participating in three trials (each trial 48 h apart): one with an unexposed observer, one with a low-amitriptyline observer, and one with a high-amitriptyline observer (order of occurrence randomised). Demonstrator fish were not exposed to amitriptyline.

Housing conditions and amitriptyline exposure

Exposure tanks were established one week prior to fish introduction. Tanks were filled with aged freshwater treated with 0.5 g L^{-1} dissolved sea salt and inoculated with 5 mL of nitrifying bacteria solution (API® Quick Start) to promote biological filtration. Throughout the experimental period, all tanks were maintained at a mean temperature of 25.4 ± 0.4 °C (range: 24.7–26.6 °C; monitored daily), pH 7.0 (monitored weekly), and under a 12:12 h light:dark cycle. Each tank contained 500 g of pearl white gravel (6–8 mm diameter) and was aerated via a 5-mm silicone air hose. Fish were fed once daily with a commercial flake food (TETRA; TetraMin Flakes), except on the day of transfer from exposure tanks to behavioural trial tanks and on the day of behavioural assays.

Observer fish were introduced to exposure tanks 24 h before exposure began. Waterborne exposure conditions were maintained in 54 tanks (30 × 22 × 20 cm; 9 L; 18 per treatment level), each housing three observer fish. To achieve the nominal amitriptyline exposure concentrations, we employed a semi-static dosing protocol in which three stock solutions were prepared in 0.5 L of reverse osmosis (RO) water: a freshwater control containing no amitriptyline (unexposed treatment condition), a low-concentration solution containing 389 µg amitriptyline hydrochloride, and a high-concentration solution containing 1831 µg amitriptyline hydrochloride (98%; Sigma Aldrich, CAS: 549-18-8; prepared by JLM). These three stock solutions were stored in opaque glass bottles at 4 °C to minimise degradation. To each 9 L exposure tank, we added 5 mL of the respective stock solution. This volume exceeds the amount required to achieve the nominal concentration based on simple dilution, as amitriptyline is known to undergo rapid initial losses from the water column through sorption to tank surfaces and organic matter following dosing (42, 62). Accordingly, initial doses were calculated using previously published water chemistry data to achieve and maintain target nominal aqueous concentrations (63). To maintain exposure levels throughout the 10-d period, we performed a 1 mL re-dose on day 5, equal to 20% of the initial concentration. This re-dosing approach was designed to offset a 20% estimated weekly loss due to degradation. Stock

solution concentrations and the dosing regimen were adapted from Manera et al. (28). To avoid confounding treatment effects with spatial variation in the exposure room, tanks were arranged in a balanced design across shelf height and room position, with all treatment groups evenly represented throughout the room. Because each exposure tank housed three observer fish, information-condition assignment following exposure was also balanced within tanks: each tank contributed at least one fish to the social-information condition and one to the combined-information condition, with the third fish allocated to preserve equal sample sizes across conditions.

To confirm exposure concentrations, a 50 mL water sample was collected from every exposure tank on days two, five, and eight of the exposure period (days are relative to the first dose administration). These samples were stored at –20 °C in 50 mL conical centrifuge tubes and a subset of 48 samples was selected for chemical analysis. This subset was balanced across treatment levels, housing rack position, and exposure days to ensure representative sampling. Water samples were analysed by the commercial environmental testing company Envirolab Services (MPL Laboratories, Perth; NATA accreditation: 2901; ISO/IEC 17025 compliant) using liquid chromatography–tandem mass spectrometry (LC-MS/MS) with a limit of quantification (LOQ) of 5 ng L^{-1} (see (28) for detailed methodology of chemical analyses).

Alarm cue preparation

Chemical alarm cues in bony fishes are released from specialised epidermal cells upon skin damage, and are detected by conspecifics as a reliable indicator of predation risk (34). In guppies, these alarm cues reliably elicit antipredator responses, such as freezing behaviour (64). Alarm cues were prepared fresh before each behavioural trial (< 15 min before) to ensure biological potency. Two adult female guppies were humanely euthanised via cold immersion: initially anaesthetised in 10 °C water for 3 min, followed by transfer into < 3 °C water for an additional 3 min to ensure complete euthanasia. Once euthanised, fish were decapitated, eviscerated, and had their caudal fins removed before being completely homogenised in 120 mL of RO water using a glass pestle. A 5 mL aliquot of this homogenate was drawn into syringes and injected into the experimental tanks as required.

Behavioural assays

Behavioural trials employed a paired-tank design in which a single observer fish was housed in a smaller focal tank positioned within a larger tank containing three unfamiliar demonstrator fish. The focal tank (25 × 10 × 23 cm) occupied one side of the larger demonstrator tank (30 × 22 × 10 cm), thereby spatially restricting the demonstrator shoal to one side of the observer's tank (Figure 1A). This configuration resulted in a single glass interface between observer and demonstrators, minimising visual distortion. The focal fish were transferred into their focal tanks along with 2 L of their respective exposure water, while demonstrator tanks contained 3 L of aged, conditioned water, consistent with the housing water used for all demonstrator fish. All fish were unfamiliar prior to testing to avoid potential familiarity effects on fear contagion (65), and were introduced and acclimated to the experimental setup for 12 h before behavioural trials commenced. All behavioural trials were conducted between 08:30 and 15:30.

Each trial consisted of two phases: a 20-min baseline activity phase, followed by a 90-min threat-information phase. The baseline phase recorded the initial activity levels of both the observer and demonstrator shoals, providing a reference for subsequent behavioural changes. The full 110-min trial was recorded from above using USB PC cameras (NEOCoolcam HD fitted with a 5–50 mm C-mount zoom lens; 1920 × 1080 resolution, 30 fps).

To start the threat-information phase, observer fish received either socially transmitted threat information or both social and personal threat information, depending on their assigned condition. In the social-information condition, a 5 mL alarm cue was discreetly introduced into the demonstrators' tank, eliciting an antipredator response from the demonstrators. Simultaneously, a 5 mL aliquot of RO water was added to the observer's tank. In the combined-information condition, both the demonstrator and observer fish received a 5 mL aliquot of alarm cue in their respective tanks. This ensured that the observer fish could detect the threat both directly through chemical cues and from socially produced information. This condition served as a maximal response comparator, allowing an assessment of how observers responded when they had direct confirmation of the threat as opposed to relying solely on social cues. All solutions were administered through a fixed tubing system behind a visual barrier to prevent unintended visual or mechanical disturbances during administration (performed by JLM, ERM, and MA). Moreover, the solutions were released under the surface of the water to avoid ripples or splashing during administration. Our design did not include a control condition in which both observers and demonstrators received water only, as this allowed us to minimise the total number of fish required, and because prior work has established that chemical alarm cues reliably elicit freezing in guppies (64) and antipredator behaviours in other fishes (reviewed in

(66). Instead, the 20-min pre-stimulus baseline period served as an informative within-individual comparison, allowing us to confirm the marked behavioural shift following threat cue administration. At the end of the trial, observer fish were removed from the experimental setup, humanely killed with an overdose of benzocaine (400 mg L^{-1}), gently blotted dry with tissue, and weighed (μ = 0.151 g, *SD* = 0.037; performed by JLM, ERM, and MA).

All videos were tracked by JLM using *IDtracker.ai* (v. 6.0.13; 67) after which the trajectories were cleaned of tracking artifacts and smoothed with a Savitzky−Golay filter (third-order polynomial with a window size of 13 frames) using *cleanRfish* (v. 2.0.0; 68) in R (v. 4.5.3; 69). One of the primary antipredator responses in guppies is freezing behaviour (i.e., prolonged immobility following threat cue delivery; 64), a pattern we also confirmed in pilot trials. To classify this behaviour objectively from continuous tracking data, we fitted a two-state (moving, freezing) hidden Markov model (HMM) using the *moveHMM* package (v. 1.12; 70). We quantified the dynamics of the fear response by using two complementary time-to-event metrics derived from these behavioural states (Figure 1B–D). Latency to resume movement was defined as the time elapsed from cue delivery until the observer first recommenced sustained movement (> 50% time spent moving within a 60-s sliding window, where the proportion of time moving was calculated continuously over time using a 60-s window advanced in 1-s increments). Recovery time was defined as the time required for behaviour to return to, and stabilise at, pre-threat stimulus (baseline) levels. This was identified using a composite behavioural similarity metric to find the point at which post-stimulus behaviour returned to pre-stimulus levels. This metric was calculated by comparing both swimming velocity and proportion of time spent moving at each second of the trial to each individual's own pre-stimulus baseline mean and standardising this difference using the pre-stimulus baseline standard deviation. The two deviation values were weighted equally and combined into a single similarity score bounded between 0 and 1, where values approaching 1 indicate that velocity and proportion of time moving were more similar to baseline levels. Recovery time was then identified as the first changepoint at which the similarity score exceeded 0.8 and remained above this threshold for the remainder of the trial, ensuring that recovery represented a sustained return to baseline-like behaviour rather than a transient fluctuation (Figure 1B–C). Changepoints were identified using the PELT algorithm (71) implemented with the *changepoint* package (v. 2.3; 72).

To quantify behavioural coupling between observers and demonstrators during post-stimulus recovery, we characterised both relative and absolute temporal alignment. First, we recorded

549 the order in which the observer resumed movement relative to the three demonstrators (ranked
550 1–4), providing a measure of relative recovery position within the group. Second, we calculated
551 the latency difference between the observer and the first demonstrator to resume movement
552 within each trial, capturing the absolute delay between the earliest available social cue of safety
553 and the observer's response. Together, these complementary metrics allow assessment of how
554 tightly observer recovery dynamics were coupled to the behaviour of demonstrator
555 conspecifics.

556 Due to logistical constraints and the need to accommodate large numbers of fish, the
557 experiment was conducted in six staggered batches. This meant that 27 trials could be run in a
558 day over two consecutive trial rounds (with up to 14 trials each). This approach ensured that
559 all fish within a batch were tested on the same day at consistent times, with treatment and
560 information condition combinations balanced across batches to maintain equal representation.
561 Although the batches were temporally staggered, they all followed identical exposure and
562 experimental protocols, and potential batch and trial (temporal) effects were accounted for in
563 the statistical analyses.

564 Camera malfunction resulted in the loss of two trial rounds (trial 1 in batch 3 and trial 2 in
565 batch 6), corresponding to a loss of 28 trials in total (9 unexposed, 11 low-exposure, and 8
566 high-exposure trials). In addition, one mortality occurred during the exposure period in batch
567 2 within the high-exposure group. Following these losses, the final sample size for the social-
568 information condition comprised 23 unexposed, 23 low-exposure, and 25 high-exposure
569 observer fish, while the combined-information condition comprised 22 unexposed, 20 low-
570 exposure, and 20 high-exposure observer fish.

571

572 *Statistical methods*

573 Behavioural states (moving, freezing) were classified from the cleaned tracking data using a
574 two-state hidden Markov model (HMM) fitted in *moveHMM* (v. 1.12; 70). To do this, tracks
575 from all focal and demonstrator fish were pooled into a single dataset and downsampled to 1-
576 s resolution before model fitting, reducing within-state serial autocorrelation while retaining
577 sufficient resolution to capture discrete freezing bouts. A single global HMM was fitted across
578 all tracks simultaneously, so that parameters were estimated from the full dataset rather than
579 from individual tracks, ensuring a common definition of behavioural states across all

individuals. Step lengths were modelled with a zero-inflated gamma distribution, accommodating the high frequency of near-zero displacements during freezing, and turn angles with a von Mises distribution, capturing the low directional persistence expected during freezing relative to moving. The most probable state sequence for each track was decoded using the Viterbi algorithm (70). Following this, the tracks and behavioural states were overlaid onto the raw videos for visual verification by JLM. Four response variables were derived from these classified tracks: latency to resume movement, recovery time, the rank order in which the observer resumed movement, and the latency difference between the observer and the first demonstrator to resume movement.

All outcomes were analysed using Bayesian generalised linear mixed-effects models (GLMMs) in R (v. 4.5.3; 69) with *brms* (v. 2.23.0; 73) and the *cmdstanr* (v. 0.9.0; 74) backend. All models included fixed effects of information condition (combined, social), amitriptyline treatment (unexposed, low, high), and their interaction, with observer body mass (scaled) as a covariate. To account for non-independence arising from repeated use of demonstrator shoals and temporal structure within the experiment, all models included random intercepts for demonstrator shoal identity and for trial round (chronological order within the day) nested within batch (chronological order across days). Latency to resume movement and recovery time were treated as time-to-event outcomes; distributional families were selected using the leave-one-out information criterion (LOOIC; 75) and posterior predictive checks. Recovery time was modelled with right-censored Weibull regression. Right-censoring accommodates observer fish that had not recovered by the end of the 90-min trial—retaining them in the likelihood via their survival probability rather than excluding them or treating the censoring bound as the true recovery time. Latency to resume movement was modelled with a right-censored hurdle lognormal model, where the hurdle component accounts for the observers that never froze (latency = 0). Baseline activity (mean proportion of time moving pre-cue) was included as an additional covariate in the latency model to account for individual differences in activity. However, it was not included in the recovery model because recovery was already defined relative to individual baseline activity. Resumption rank was modelled with cumulative-logit ordinal regression. The observer–demonstrator latency difference (observer latency minus minimum demonstrator latency) was modelled with a Gaussian distribution. Measured water amitriptyline concentrations were modelled separately with a Gamma distributed GLMM (log link), with fixed effects of amitriptyline treatment (unexposed, low, high), day since dose (count of days since the last dose), and their interaction, and a random

intercept for batch. All models were fitted with four chains of 6,000 iterations (4,000 warm-up, adapt delta = 0.95, max treedepth = 12) and chain convergence was confirmed via $\hat{R} < 1.01$. We report posterior marginal means and pairwise contrasts (average marginal effects) with 95% credible intervals, estimated via the *marginaleffects* package (v. 0.32.0; 76). Evidence strength is summarised as the probability of direction (*pd*) calculated with *bayestestR* (v. 0.17.0; 77); contrasts with $pd > 0.90$ are interpreted as providing evidence of a meaningful directional difference.

All analyses were conducted blind to amitriptyline treatment and information condition. Blinding was implemented using a randomly generated coding script that concealed group identities during model specification and selection, with group identities only having been revealed after the final models had been selected.

Author contributions

Following the Method Reporting with Initials for Transparency (MeRIT) guidelines (78), we have reported what processes were conducted by certain members of the author team, to improve the granularity of author contributions as well as to enhance reproducibility and replicability (see Materials and Methods). Here, we report a list of each author's roles using the Contributor Role Taxonomy (CRediT; 79). **Jack L. Manera:** Conceptualisation, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Visualisation, Writing–Original Draft Preparation, and Writing–Review & Editing. **Eleanor R. Moore:** Investigation, Project administration, Writing–Review & Editing. **Mirjam Amcoff:** Investigation, Project administration, Resources, Writing–Review & Editing. **Bob B.M. Wong:** Conceptualisation, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, and Writing–Review & Editing. **Jake M. Martin:** Conceptualisation, Project Administration, Supervision, and Writing–Review & Editing. **Niclas Kolm:** Conceptualisation, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, and Writing–Review & Editing. **Michael G. Bertram:** Conceptualisation, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, and Writing–Review & Editing.

Data availability

All data and code (R script) used to produce this work are publicly available on J.L. Manera's GitHub repository (https://github.com/JLManera/26-ami_social_fear), as well as via the OSF framework (https://osf.io/t9pu6/overview?view_only=b6315e91268d4295ac29b330a1bfc5b7). To promote transparent and complete reporting, we followed the EthoCRED reporting recommendations for behavioural ecotoxicity studies (80).

Conflicts of interest

The authors declare that they have no competing interests.

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