Short- and Long-Term Habituation of Photonegative and Exploratory Responses in the Flatworm Planaria (*Dugesia*)

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Two experiments address the habituation of photonegative and exploratory responses in the flatworm planaria (*Dugesia*). Planarians possess a well-documented photonegative response; Experiment 1 showed that repeated exposures to a bright light source with short inter trial intervals (ITIs) within 1 experimental session gradually weakens the unconditioned photonegative response. In addition, it was found that presentation of an unexpected arousal-increasing stimulus (dropped water or a shock) temporarily re-establishes the photonegative response. Experiment 2 addressed the development of long-term habituation; we recorded the locomotor activity of the animals exposed to an inescapable bright light. Experiments 2A and 2B showed that planarians develop long-term habituation but only when they were trained in relatively novel contexts; when they were trained in familiar contexts (in surfaces similar to the ones in the home) the development of habituation was severely impaired. The results are discussed by reference to the theory of short- and long-term habituation put forward by Allan R. Wagner (Wagner, 1976), highlighting the impact that this theory has had in the research of invertebrate learning.

Keywords: short-term habituation, long-term habituation, invertebrate learning, planaria

In the 1970s, after the publication of the momentous Rescorla-Wagner model (Rescorla & Wagner, 1972; Wagner & Rescorla, 1972), Allan R. Wagner proposed an integrative theoretical framework for animal learning which was consistent with the burgeoning information processing paradigm in human memory (e.g., Atkinson & Shiffrin, 1968). Wagner's proposal allowed researchers to address nonassociative phenomena, like short-term habituation, and associative phenomena, like long-term habituation and Pavlovian conditioning, applying a few well-established general principles derived from research on human memory and animal associative learning (Wagner, 1976).

Wagner's approach to learning and memory can be summarized as a system involving a limited in capacity information processor (short-term memory, STM) that processes information incoming from the environment; this allows representations of these events and their relationships to be encoded in a high capacity long-term memory store. The information coming from the animal's surroundings can be activated in the short-term store in different ways. An unexpected stimulus will typically result in full activation of its representation in the information processor; Wagner refers to this activation as A1 state. This type of activation ensures high processing, the establishment of an accurate memory of the event in the long-term store, and strong command on the behavior of the individual. Decay from A1 leads to a marginal activation of the stimulus, or A2 state; activation in A2 involves lessened processing and lower control of the response system. Key elements of the Wagnerian system are that stimuli can be indirectly activated in the marginal A2 state by an event with which an association has been previously established; and that a stimulus already activated in A2 cannot be activated in A1—even if the stimulus is physically presented again.

This relatively simple set of principles can account for a wide range of nonassociative and associative phenomena. Wagner and his collaborators applied those principles to the analysis of the exposure effects of stimuli upon the unconditioned response they elicit. It is well established that repeated presentations of an eliciting stimulus (e.g., a sudden noise that results in a startling response) gradually weakens the response to the stimulus. This habituation effect has been the focus of extensive research for decades, and a highly well-articulated set of 10 characteristics of habituated responses has been described (Rankin et al., 2009; Thompson & Spencer, 1966). One such characteristic is that "more frequent stimulation results in more rapid and/or more pronounced response decrement, and more rapid spontaneous recovery . . ."

(Rankin et al., 2009, p. 137). This suggests not only that a recent presentation of the eliciting stimulus is more likely to reduce the responsiveness of the subject to the event, but also that this reduced responsiveness is sensitive to the passage of time; this characterizes this particular example of habituation as short-term habituation. In Wagnerian terms, recent presentation of the eliciting stimulus results in its elements being activated (or primed) in the marginal A2 state in the information processor; as mentioned above, activation in A2 prevents full activation (A1) of the event. This self-generated priming mechanism elegantly accounts for short-term habituation (see Whitlow, 1975).

Another characteristic of short-term habituated responses is that they are vulnerable to distraction. This dishabituation phenomenon (characteristic 8 of habituation; Rankin et al., 2009) shows that the reduction in the magnitude or rate of the unconditioned response is not the consequence of fatigue or sensory adaptation. Wagner's theoretical approach smartly predicts dishabituation: presentation of an unexpected distracting stimulus displaces the representation of the eliciting stimulus from the limited in capacity short-term information processor. This allows the subsequent presentation of the target stimulus to be fully activated in A1, resulting in the unconditioned response reappearing also in full strength (Whitlow, 1975).

Spaced (with longer ITIs) presentations of the eliciting stimulus across days, although less effective in reducing the magnitude or frequency of responses, result in habituation that is less vulnerable to the passage of time (e.g., Davis, 1970). This long-term habituation follows, according to Wagner, a different route than the short-term habituation. While short-term habituation can be said to be the consequence of self-generated prime (by presentations of the eliciting stimulus close in time), long-term habituation obeys to retrieval or associative generated priming. Presentations of the eliciting stimulus lead to the establishment of an association of the stimulus with the experimental context—that plays the role of a standard conditioned stimulus (Urcelay & Miller, 2014). Exposure to the context associatively activates a representation of the eliciting stimulus in A2, preventing the full expression of the natural response to the stimulus. Wagner and his collaborators presented convincing evidence for context-dependent associative long-term habituation. For example, after habituation training, additional presentations of the contextual cues alone (an extinction procedure) reinstated the strength of the response to the eliciting stimulus compared with a control group in which the context retained its predictive value (see Wagner, 1976, p. 122).

In the early 80s, Allan Wagner was part of a discussion group on how comparative learning studies would help us ascertain whether different groups of animals learn according to the same rules or, on the contrary, learning has independently evolved in different animal phyla (Menzel et al., 1984). The group report pointed out that learning theory traditionally assumed that "the empirical laws of Pavlovian and Thorndikian conditioning as discovered in the laboratory investigations with such conventional subjects as dog and rat, could serve as the underline axioms of a theory, capable of deducing all instances of learning as properly defined" (Menzel et al., 1984, p. 252). According to the authors, a limitation of this approach is that an important instance of behavioral plasticity investigated in invertebrates, habituation, would not be admitted as true (associative) learning. This potential problem becomes irrelevant, however, by applying an integrative approach as the one proposed by

Wagner (e.g., Wagner, 1976) that considers habituation to be just another learning phenomenon that emerges from the application of a few well-articulated general principles.

Wagner's work has had a huge impact on the research of learning in invertebrate models. Examples of this influence can be traced back to the 1990s, when work on the crab *Chasmagnathus* showed evidence of short- and long-term habituation of the escape response to a visual danger stimulus. After 15 presentations of a danger sign (an opaque screen moving overhead the crabs), a decrement in the escape response was observed; this weakening of the response was retained for several days (Pedreira, Dimant, Tomsic, Quesada-Allue, & Maldonado, 1995). The observed long-term habituation was reliant on associative learning as predicted by Wagner (1976): the development of an association between the experimental context and the visual danger signal presumably primed a representation of the sign in the presence of the contextual cues, weakening the unconditioned response it elicited. Subsequent assessment of the long-term habituation showed that exposure to the experimental context for 2 hr before the habituation training impaired the development of habituation (the animals kept responding to the visual danger signal in comparison to a group of animals nonpre-exposed to the context). Also, animals tested in a context different to the one used for habituation training failed to show long-term habituation, revealing context dependence of habituation as predicted by Wagner (Tomsic, Pedreira, Romano, Hermitte, & Maldonado, 1998). Similar results have been observed in the nematode worm *Caenorhabditis elegans*. Worms swim back for some distance in response to the vibrations produced by a mechanical tap in the side of the container where they swim—a tap-withdrawal response. Short-term habituation of this response can be observed with massed presentations of the tap stimulus with short ITIs; with longer ITIs, long-term habituation is observed (Beck & Rankin, 1997). Furthermore, prior exposure to the context, exposure to the context after habituation training, and a change of context between training and test all result in impaired long-term habituation (Rankin, 2000). These results are all consistent with Wagner's theory of long-term habituation as an associative process that requires the existence of a link between the experimental context and the eliciting stimulus.

We report here original work with the goal of updating our knowledge on the habituation processes that can be observed in the flatworm planaria (*Dugesia*). The planaria, a nonparasitic flatworm, has been widely used in developmental biology and behavioral research. It has been used as a fruitful model for stem cell biology (e.g., Rink, 2013), regeneration (e.g., Elliott & Sánchez Alvarado, 2013; Inoue et al., 2004), toxicology (e.g., Grebe & Schaeffer, 1991), as well as in studies on the evolution of the nervous system (e.g., Nakazawa et al., 2003) and cognition (e.g., Alvarez, Loy, & Prados, 2017) among others. Its nervous system presents structural and physiological similarities to the nervous system of vertebrates: centralized and bilateral with similar neural networks, transmitters, and neuromodulators (Buttarelli, Pellicano, & Pontieri, 2008). It also exhibits complex learning in standard Pavlovian and instrumental conditioning tasks (e.g., Lee, 1963; Prados et al., 2013) and displays behavioral responses to drugs of abuse that are similar to those seen in mammals, including cocaine behavioral sensitization (Rawls, Patil, Yuvasheva, & Raffa, 2010) and conditioned place preference (Amaning-Kwarteng, Asif-

Malik, Pei, & Canales, 2017; Hutchinson, Prados, & Davidson, 2015; Mohammed Jawad, Hutchinson, & Prados, 2018).

The planaria became a popular model for learning research in the 1950s and 1960s of the last century and although there is a vast literature on conditioning (e.g., Prados et al., 2013), little has been published on habituation. Evidence for short-term habituation can be traced back to the early years of the 20th century, when Walter showed that rotation of the aquarium in which planarians were housed produced a momentary halt in the sliding of the animals; repeated rotations progressively weakened the halting; however, after a 1-min interval the halting response spontaneously recovered (Walter, 1907). Other studies showed that animals' natural responses to vibration (Dilk, 1937) and changes in luminance (Miller & Mahaffy, 1930) readily habituated. The habituation of the longitudinal contraction in response to a drop of water on the body of the animal has been widely used to demonstrate short-term habituation in the planaria; this observation has become a popular high-school lab demonstration (see, e.g., Owren & Scheuneman, 1993).

When looking for examples of long-term habituation in planarians the paucity of evidence is overwhelming. Westerman (1963) exposed planarians to an intense 3-s light stimulus 25 times per day (inter trial interval, ITI = 30 or 60 s) over 16 days; the longitudinal contraction or shrinking response of the animals in response to the light stimulus was recorded. The results showed a reduction in the percentage of animals that showed the response over the first habituation training session (from more than 30% in the first block of five trials to less than 20% in the final block of trials). The results of the 16th training sessions showed that about 25% of the animals showed the shrinking response during the first block of five trials (indicating that there was some level of spontaneous recovery of the response 24 hr after the last training session); however, this percentage went down to near zero during the second block of training trials. This strongly suggests a net effect of experience with the light stimulus that can be considered an example of long-term habituation. Despite these early demonstrations of short- and long-term habituation, systematic investigations on habituation in flatworms have apparently never been performed (Jacobson, 1963). More than 50 years later, this statement can still be said to be true.

The planarian flatworm is one of the most primitive animals to develop two forward-facing eyecups, each composed of photoreceptors and pigment cells in a rhabdomeric structure registering the presence and direction of light. Planarians exhibit negative phototaxis in response to the light exposure, traveling away from it to seek cover. Negative phototaxis has been shown to be dependent on the intensity of the light-stimulus. Davidson et al. (2011) assessed the response of planarians to different intensities of white light. The animals exhibited a graded, sigmoidal, photonegative response to light intensity: a two-octave increase in luminance (on the upward slope of the sigmoid) corresponded to a 9% increase in the speed planarians traveled to avoid light. Using a similar experimental setting, Paskin, Jellies, Bacher, and Beane (2014) reported photophobic responses to a range of wavelengths; short wavelength (ultraviolet, blue, and green) and white lights produced intense negative phototaxis. However, longer wavelengths (red light) produced no effect whereas an infrared light was found to be slightly attractive for the planaria.

In the experiments reported here, we assessed whether repeated exposures within a single experimental session to white light results in habituation of the photonegative response. Experiment 1 was designed to assess short-term habituation and dishabituation. Experiment 2 addressed the establishment of long-term habituation (by training the animals over multiple daily sessions) and the role of the context in the development of habituation.
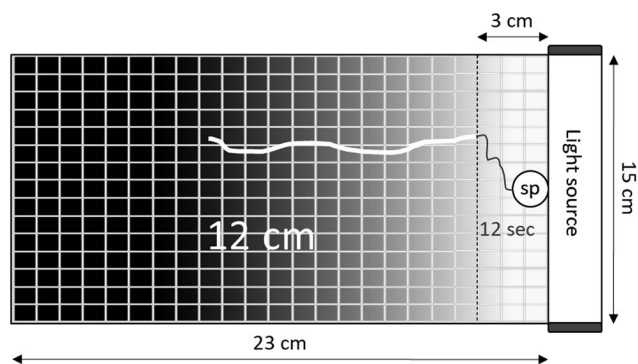
Experiment 1

Experiment 1A

In this experiment, we made use of a similar preparation to the one used by Davidson et al. (2011) and Paskin et al. (2014). Animals were placed in a container close to a white light source and their photonegative response (swimming away from the light source) was measured over multiple trials within a day.

Method.

Subjects. Thirteen brown planarians (*Dugesia*) purchased from Blades Biological Ltd. (Kent, U.K.) served as the subjects in the present experiment. The animals were held in a plastic container $20 \times 30 \times 20$ cm filled with 5 L of tap water treated with 0.5 ml/L AquaSafe (Tetra, Melle, Germany). AquaSafe is a conditioner that makes tap water safe for fish and other freshwater creatures; it neutralizes chlorine, chloramines, and heavy metals present in tap water that can be harmful to the animals. The planaria colony was kept at a room temperature of 20 °C (± 2) with light cycle of 14:10 light:dark hours. The animals were fed boiled egg white twice per week for 1–2 hr; the water of the aquarium was changed after feeding the animals. The animals were assigned at random to one of two groups: Experimental ($n = 7$) and Control ($n = 6$).

Apparatus. A rectangular tank $23 \times 15 \times 9$ cm made of transparent polymethyl methacrylate (PMMA) was used as the experimental setting for the present experiment. A graded sheet under the tank divided in 15×23 1-cm squares was used to determine the position of the flatworms during the experimental sessions. One of the 15 cm walls was attached to the light source (a T5 4W fluorescent tube 15 cm long powered by four AA batteries); this side was designated as the start area (3 cm from the light source) for the experimental sessions. At the onset of each trial, the animal was placed in the center of the starting area close to the wall, the starting point (see Figure 1). The tank was filled to a depth of 0.5 cm with water treated with AquaSafe (175 ml approx.).

Procedure. The animals were transferred from the colony to the experimental tank and allowed to explore the new environment for 5 min before the testing started. There were two phases in the experiment: habituation and dishabituation. Both phases took place within a single session that lasted approximately 30–45 min for each animal—that was tested individually. For the animals in the Experimental group, the first phase consisted of 10 trials of habituation to the light stimulus. Each trial started by gently placing the animal in the start point of the tank (see Figure 1), 5 s before the light was switched on. We measured the time that took the animal after the presentation of the light to reach the line that limited the start area (3 cm). From that moment onward, we measured the distance the animal covered during 60 s moving away from the light source. Both measures (latency to leave the start area and the

Figure 1. Schematic representation (from above) of the apparatus used in Experiment 1. The discontinuous line indicates the edge of the starting area. The continuous lines illustrate the trajectory followed by one animal during a particular trial (indicatory of the photonegative response). In this particular case, the animal would have taken 12 s to leave the start area (black line), and would have covered 12 cm in the direction opposite to the light source (white line). sp = start point.

distance traveled away from the light source) are indicative of the innate photonegative response of the planaria. Sixty seconds after the animal left the starting area the light was switched off and the animal was left wherever it was at the end of the trial for 30 s before a new trial started.

After the tenth habituation trial, the dishabituation phase of the experiment started. As during habituation, the animal was placed in the starting point; however, an unexpected stimulus was presented: four drops of water were released from 1 cm above the animal. This unequivocally results in a shrinking response, indicating that the animal is sensitive to this particular stimulation. Five seconds after the four drops were administered, the light was switched on again and the photonegative response was measured. This test trial was followed by three more habituation trials. The Control group was treated in exactly the same way across the different phases of the experiment but the light stimulus was never presented.

Data analysis. The analysis of photonegative responses throughout this article was conducted with analyses of variance (ANOVA) using a rejection criterion of $p < .05$. We used SPSS to run all the analyses. The reported effect size for ANOVA with more than one factor is partial eta squared (η_p^2), while for comparisons between two means it is eta squared (η^2). For both measures of effect size, 95% confidence intervals (CIs) were computed using the method reported by Nelson (2016).

Results and discussion.

Habituation. Figure 2A (left panel) shows the group average latency to leave the starting area throughout two blocks of five habituation-training trials (B1, T1-T5; and B2, T6-T10). A mixed ANOVA with Group (Experimental vs. Control), Blocks of Trials (B1 and B2) and Trials as factors showed significant effects of Group, $F(1, 11) = 15.70$, $p < .01$, $\eta_p^2 = .58$, 95% CI [.11, .75], Blocks of Trials, $F(4, 44) = 28.81$, $p < .01$, $\eta_p^2 = .72$, 95% CI [.37, .82], and a significant Group × Blocks of Trials interaction, $F(1, 11) = 6.40$, $p = .02$, $\eta_p^2 = .36$, 95% CI [.02, .58]. The remaining factors and interactions were all nonsignificant, maximum $F(4, 44) = 1.37$. Further analyses were carried out to analyze the Group × Blocks of Trials interaction (simple main effects). A Repeated Measures ANOVA with the Blocks of Trials on the data of Group Experimental showed a significant effect of Blocks, $F(1, 6) = 39.36$, $p < .01$, $\eta_p^2 = .86$, 95% CI [.47, .91]; the same analysis on the data from Group Control showed a nonsignificant effect of Blocks of trials, $F(1, 5) = 3.19$. Furthermore, the two groups differed in the first Block of trials, $F(1, 11) = 18.09$, $p < .01$, $\eta_p^2 = .62$, 95% CI [.22, .75], but not in the second block of trials, $F(1, 11) = 4.54$.

Figure 2B (left panel) shows the group average distance covered by the animals away from the light over 60 s after they left the starting area throughout two blocks of five habituation-training trials (B1, T1-T5; and B2, T6-T10). A mixed ANOVA with Group (Experimental vs. Control), Blocks of Trials (B1 and B2) and Trials as factors showed significant effects of Group, $F(1, 11) = 8.69$, $p < .01$, $\eta_p^2 = .44$, 95% CI [.02, .67], Blocks of Trials, $F(4, 44) = 15.87$, $p < .01$, $\eta_p^2 = .59$, 95% CI [.19, .73], and a significant Group × Blocks of Trials interaction, $F(4, 44) = 5.17$, $p = .04$,
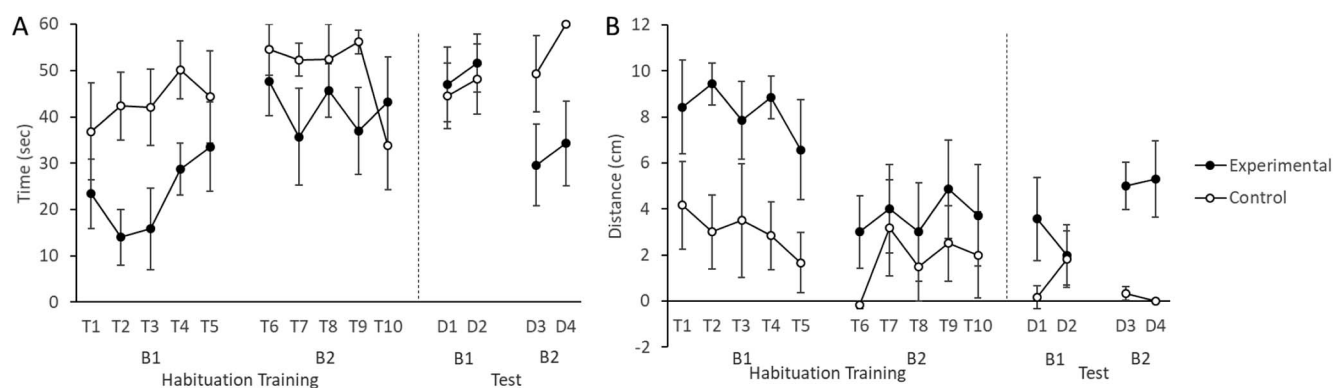
Figure 2. Results of Experiment 1A. (A) Group average latency (±*SEM*) to leave the starting area throughout the 10 trials of habituation training (T1–T10) and the dishabituation test trials (D1–D4). (B) Group average distance covered by the animals away from the light (±*SEM*) over 60 s after they left the starting area throughout the 10 trials of habituation training (T1–T10) and the dishabituation test trials (D1–D4).

$\eta_p^2 = .32$, 95% CI [.00, .55]. The remaining factors and interactions were all nonsignificant, Fs < 1. Further analyses were carried out to analyze the Group × Blocks of Trials interaction (simple main effects). A Repeated Measures ANOVA with the Blocks of Trials on the data of Group Experimental showed a significant effect of Blocks, $F(1, 6) = 34.52$, $p < .01$, $\eta_p^2 = .85$, 95% CI [.44, .90]. The same analysis on the data from Group Control showed a nonsignificant effect of Blocks of trials, $F < 1$. Furthermore, the two groups differ in the first Block of trials $F(1, 11) = 15.19$, $p < .01$, $\eta_p^2 = .58$, 95% CI [.18, .73]; but not in the second block of trials, $F(1, 11) = 1.68$

Dishabituation test. Figure 2A (right panel) shows the group average latency to leave the starting area by blocks of two dishabituation test trials (B1, D1–D2 and B2, D3–D4). A mixed ANOVA with Group (Experimental vs. Control), Blocks of Trials (B1–B2) and trials as factors showed a significant effect of Group, $F(1, 11) = 6.40$, $p = .02$, $\eta_p^2 = .36$, 95% CI [.02, .58], and a significant Group × Blocks of Trials interaction, $F(1, 11) = 6.43$, $p = .02$, $\eta_p^2 = .37$, 95% CI [.02, .69]. The remaining main factors and interactions were all nonsignificant, maximum $F(1, 11) = 1.09$. Analysis of the Group × Blocks interaction (simple main effects) revealed a group difference only in the second block of test (B2), $F(1, 11) = 21.83$, $p < .01$, $\eta_p^2 = .66$, 95% CI [.28, .78]. Figure 2B (right panel) shows the group means for the distance covered by blocks of two dishabituation test trials (B1, D1–D2 and B2, D3–D4). A mixed ANOVA with Group (Experimental vs. Control), Blocks of Trials (B1–B2) and trials as factors showed a significant effect of Group, $F(1, 11) = 8.41$, $p = .01$, $\eta_p^2 = .43$, 95% CI [.05, .63], and a significant Group × Blocks of Trials interaction, $F(1, 11) = 6.32$, $p = .02$, $\eta_p^2 = .36$, 95% CI [.02, .58]. The remaining factors and interactions were all nonsignificant, maximum $F(1, 11) = 1.51$. Analysis of the interaction (simple main effects) revealed a group difference only in the second block of test (D2), $F(1, 11) = 23.34$, $p < 01$, $\eta_p^2 = .68$, 95% CI [.30 .79].

The present results show clear evidence of habituation of the photonegative response. The Control group, exposed to the same experimental context but without any exposure to the light, initially took much longer to leave the starting area and swam shorter distances from the start point than the animals in the Experimental group, exposed to the white light. However, repeated exposures to the light gradually increased the latency to escape from the area immediate to the light source and reduced the distance they swam from the light.

The decrease in the activity of the animals in the presence of the light could be attributed to physical fatigue or sensory adaptation to the light stimulus. However, presenting the animals with a new, unexpected stimulus (drop of water) decreased the latency to escape from the starting area and increased the distance the animals traveled from the light stimulus. The activity in the control group, however, was unaffected by the presentation of this unexpected stimulation. The effect of the unexpected stimulation in the Experimental group was obvious only in a second block of two dishabituation test trials. This was unexpected; perhaps the drop of water and the automatic shrinking response it elicits interferes with the locomotor activity that allows the animals to swim away from the light; according to this, the dishabituation effect could be masked during the initial test trials. The results showed that, in the second block of trials, animals in the experimental group took less time to leave the starting area and covered more distance from the

light that those in the control condition. The experiment, therefore, successfully discards physical fatigue or sensory adaptation as the cause for the reduced responsiveness to the light: if anything, accumulating extra trials should have produced more fatigue or adaptation. In this experiment, the animals were tested immediately (5 s) after the presentation of the unexpected stimulus. To avoid interference with the photonegative response, we could allow a longer period of recovery after the stimulation. In Experiment 1B we assessed this possibility by testing the animals 60 s after the presentation of an unexpected (i.e., dishabituating) stimulus. In addition, we wanted to increase the generality of the dishabituation effect observed in Experiment 1 by using a different dishabituating stimulus, an electric shock.
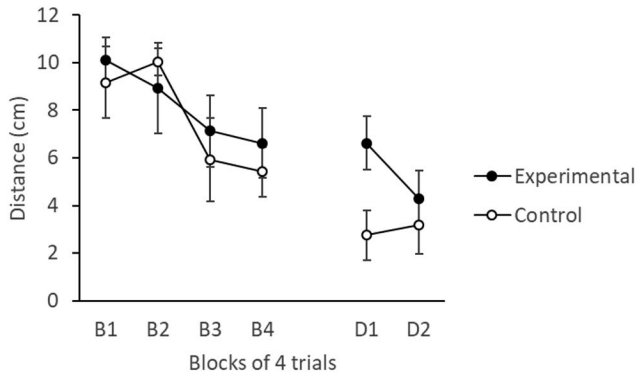
Experiment 1B

Experiment 1B had the goal to further explore habituation and dishabituation of the negative phototaxis response in planarians. Two groups of animals (Experimental and Control) received habituation training following the procedure used in Experiment 1A. After habituation of the photonegative response, animals in the experimental group were exposed to a brief electric shock; after a 60-s recovery interval, the photonegative response was tested again. Our hypothesis was that presentation of a brief shock (that elicits a characteristic shrinking response) could interfere with the expression of the photonegative response. Testing the animals 60 s after this stimulation would increase the chances of observing a dishabituation effect in the first test trials leading then to the expected immediate (re)habituation of the response to the light.

Method.

Subjects and apparatus. The subjects were 12 brown planarians; the animals were assigned at random to one of two groups: Experimental ($n = 6$) or Control ($n = 6$). The apparatus and stimuli used were the same described above for Experiment 1A.

Procedure. The procedure of Experiment 1B replicates the procedural details of Experiment 1A, except that both groups (Experimental and Control) received habituation training to the light for 16 trials. After the 16th trial, animals in group Experimental were presented with an unexpected stimulus: a 0.5 s shock delivered by presenting two electrical wires in front of the head and behind the tail that were connected to the anode and cathode of a 9 V battery. The shock resulted in a characteristic response of shrinking (see, for example, Prados et al., 2013). Animals in the group Control were handled in the same way as those in the Experimental group, but no shock was delivered (the wires were not attached to the battery). Sixty seconds after the presentation of the shock, the animals were given eight additional trials of habituation.

Results and discussion. Figure 3 shows the distance covered by the animals away from the light over 60 s (timed from the moment they left the starting area) during four blocks of four habituation trials (B1–B4); and the distance covered during the subsequent eight trials after the presentation of the shock to the animals in the experimental group, a dishabituation test. A mixed ANOVA with Group (Experimental vs. Control) and Blocks of Trials (B1–B4) as factors conducted on the habituation data showed a significant effect of Blocks of Trials, $F(3, 30) = 9.30$, $p < .01$, $\eta_p^2 = .48$, 95% CI [.16, .61]. Neither the Group factor nor the Group × Blocks of Trials interaction was significant, Fs < 1.

Figure 3. Results of Experiment 1B. Group average distance (±*SEM*) covered by the animals away from the light over 60 s after they left the starting area by blocks of four trials during the habituation training (B1–B4) and the dishabituation test (D1–D2).

A mixed ANOVA with Group and Blocks of Trials carried out on the dishabituation test data showed a significant Group × Blocks of Trials interaction, $F(1, 10) = 5.98$, $p = .03$, $\eta_p^2 = .37$, 95% CI [.00, .63]. Neither of the main effects was significant, largest $F(1, 10) = 2.90$. Further analyses (simple main effects) were carried out to analyze the Group × Blocks of Trials interaction. The two groups significantly differed during the first block of test trials, $F(1, 10) = 6.34$, $p = .03$, $\eta^2 = .38$, 95% CI [.00, .64]; the groups did not differ during the second block of test trials, $F < 1$.

The present results replicate the results of Experiment 1A: animals repeatedly exposed to the white light source showed evidence of habituation of the photonegative response. After the habituation training, the animals were presented with a nonexpected shock stimulus that produces a characteristic shrinking response. In this case, instead of waiting for a mere 5 s (as in Experiment 1A) we allowed the animals to recover for 60 s. After this, the animals showed a restored photonegative response (compared with the animals in the control group that were not shocked). This is indicative that the reduced photonegative response observed because of repeated exposures to the light stimulus could not be attributed to mere fatigue or sensory adaptation but to genuine habituation. This example of habituation would most likely be short-term habituation: the animals were given relatively massed trials with the light within one single session. Experiment 2 addressed the establishment of long-term habituation by training animals over multiple daily sessions.
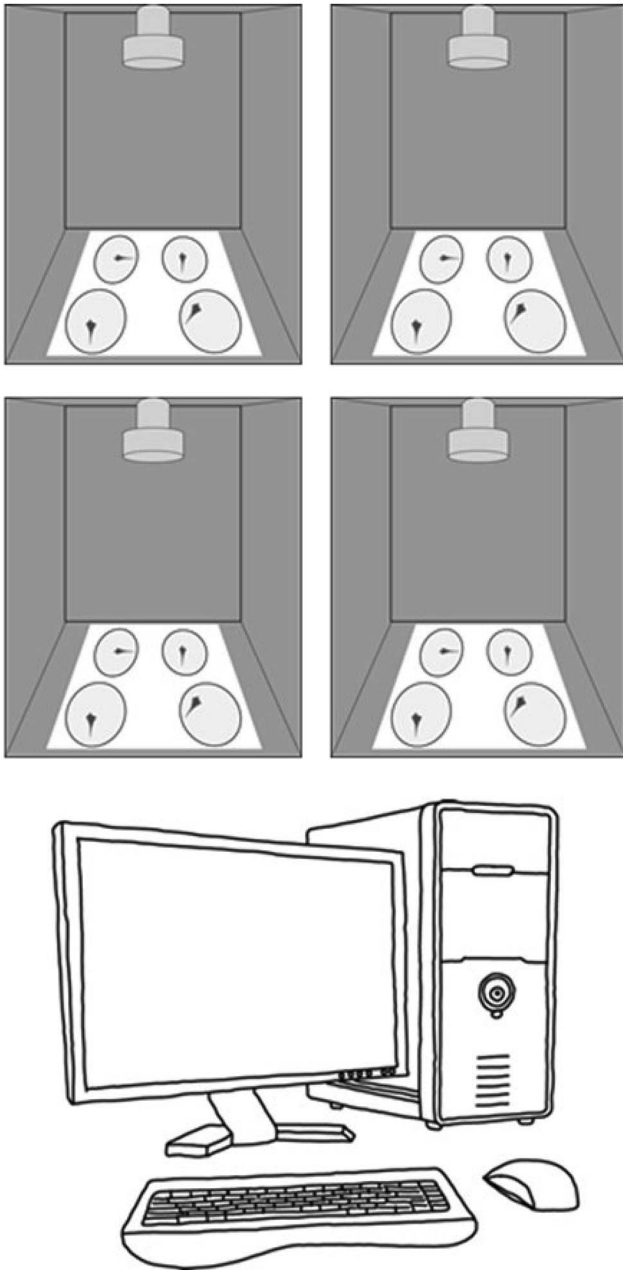
Experiment 2

Despite the popularity of a habituation preparation in planarians (e.g., Owren & Scheuneman, 1993), near to no attention has been paid to its temporal course. The only study that addressed the effect of repeated presentations of an eliciting stimulus over several days and tested the animals reactivity to the stimulus after an interval of at least 24 hr is that reported by Westerman more than 50 years ago (Westerman, 1963). In this experiment, the author monitored the shrinking response to the sudden presentation of an intense light (1370 lx); the light stimulus was presented 25 times a day for 16 days. Ten planarians (out of 36) that showed about 30 responses in their first 50 trials, were then fully habituated and

displayed zero shrinking responses in the last 50 trials during the last 2 days of habituation training. These animals still showed a significant reduction in the display of the shrinking response 3 weeks (average of 12% responses) and 7 weeks (average of 16% responses) after they reached the criterion (zero responses over 50 trials). Thus, this report suggests long-term habituation to light stimulation in the planaria.

Unfortunately, Westerman did not assess the role of the contextual cues in the development of long-term habituation. This is an important question given the claim that invertebrates are not sensitive to environmental contexts, which was put forward in a discussion of latent inhibition in invertebrates (Lubow, 2010). We have recently gathered evidence that planarians are sensitive to different surfaces that are part of the environmental context (i.e., a petri dish). Specifically, Mohammed Jawad et al. (2018) reported a series of experiments assessing the development of conditioned place preferences using a 10% sucrose solution as the reinforcer. Animals developed conditioned place preferences associating different surfaces (smooth or sandy) with the unconditioned effects of sucrose. The natural reaction to sucrose, a decrease in the locomotor activity of the animals, weakened with experience. Although this outcome could be considered an instance of habituation, the animals were found to display a hyperactivity conditioned compensatory response in the presence of the contextual cues alone; this led the authors of the study to consider it as an example of tolerance development (to sucrose) similar to what has been documented in rodents (e.g., Siegel, 1975). The experiments by Mohammed Jawad and colleagues suggest that planarians can effectively associate contextual cues with a physiological stimulus diminishing the impact the stimulus has on the organism. In terms of Wagner's theory of long-term habituation, the contextual cues could prime a representation of the eliciting stimulus (sucrose) into A2 state, weakening their processing and control over the behavior of the individual. The experiments reported below assessed this possibility investigating long-term habituation in planarians.

In preliminary experiments carried out in our laboratory, planarians were placed in petri dishes where they could be exposed to light from underneath (see Figure 4). The light stimulus could be presented intermittently over a 1-hr session, allowing us to measure the locomotor activity to the context and the light stimulus; in other cases, the light was presented continuously over 1-hr sessions. In both cases, locomotor activity decreased during a single habituation training session (that could be taken as an example of short-term habituation). However, we did not find evidence of long-term habituation in experiments in which several daily sessions were carried out.

One aspect that we thought at this point could be of relevance was the familiarity of the contextual cues. One feature of the experimental contexts that has been found to be effective to serve as a conditioned stimulus is the nature of the surface. In the experiments by Mohammed Jawad et al. (2018), for example, a significant effect of context was found when the animals were exposed to smooth and rough contexts (the plastic or glass of petri dishes, on the one hand; and a sandy surface on the other). The planarians used in the preliminary experiments referred to above were housed in a plastic container with a smooth surface; we hypothesized that, because of the familiarity of the animals with smooth surfaces, the smooth experimental context was similar to the home environment, making it difficult to establish

Figure 4. Schematic representation of the apparatus used in Experiments 2A and 2B. Each box contained four petri dishes allowing the recording of the activity of four animals (see text for full details). We could test up to 16 animals simultaneously; the recordings were stored in a computer hard disk and subsequently analyzed using a video-track software.

context-light associations and hence reducing the prospect of observing long-term habituation.

Experiment 2 had as goal to identify a multiday training procedure to assess the establishment of long-term habituation—that shows retention over a 24-hr retention interval. A second motivation for these experiments was to explore the role of the contextual cues in the development of long-term habituation. In Experiment 2A we used two different surfaces as the contextual cues: on the

one hand, a familiar smooth context, very similar to the housing context of the animals; on the other hand, a sandy context that could be more distinctive for the animals. Our hypothesis was that exposure to the light in the novel (perhaps more salient) context could induce the development of long-term habituation of the locomotor activity of the planaria. Animals were assigned to two groups, Smooth and Sand. The group Smooth was given habituation training in the smooth context similar to the housing context with a relatively dim light (45 lux); they were then tested in the novel sandy context. Animals in the Sand group were given habituation training and test in the sandy context.

Experiment 2A

Method.
Subjects and apparatus.   The subjects were 48 brown planarians kept under the same conditions described in Experiment 1. The animals were taken from the colony and housed individually 3 days before the beginning of the experiment in plastic ice cube trays where they were kept throughout the experiment. The animals were assigned at random to one of two experimental groups: Smooth ($n = 23$) and Sand ($n = 22$). The final number of animals is less than the 24 originally allocated to each Group because three animals died over the course of the experiment. Animals were tested in groups of up to 16 by using four wooden boxes that would each hold four dishes. Each box contained a dimmable 30×30 cm 24W LED panel; in the present experiment, the light was set at 45 lux. The dishes were placed on top of the LED panel. A camera on the top center of the wooden box could simultaneously record the activity of four animals using SharpCap capture software; these videos were subsequently analyzed using a video-track system (ViewPoint, Lyon, France) allowing us to register the activity of the four animals in each box during the experimental sessions (see Figure 4).

Animals were tested in 10 cm in diameter watch glass soda lime dishes. One was a glass dish whereas the second one was a glass dish divided in eight identical areas, four of which were covered with sand (glued to the glass with white silicone); the glass and sand areas were intermixed providing a "star" configuration. We refer to the glass dish as the Smooth context, and the star sandy one as the Sand context. The animals were exposed to a constant light of 45 lux throughout the experimental sessions.

Procedure.   The experiment was carried out in three identical replications. There were two phases in this experiment: habituation (Days 1–5) and test (Day 6). During the habituation training, animals in the group Smooth were placed for 1 hr in the smooth context; animals in the Sand group were exposed to the Sand context. The animals could freely move and we recorded the distance covered within the dish during the 1-hr session. After each experimental session, the planarians were returned to their home ice cube trays. On the sixth day of the experiment, a test trial took place in which both groups of planarians were exposed to the Sand context for an hour. We expected the locomotor activity of the animals to decrease over the habituation training in both contexts; exposure to the Sand context during the test was expected to reveal habituation in the group previously exposed to this context; the group Smooth, which was equally handled but exposed to an alternative context, should show higher levels of locomotor activity. Lower levels of activity in the group trained and tested in the

Sand context would be evidence of long-term habituation of the exploratory response elicited by the novel context.

Results and discussion. Figure 5A displays the mean locomotor activity (centimeters/minute) of the animals in the experimental groups over the 5 days of habituation training. A Repeated Measures ANOVA with Group (Smooth vs. Sand) and Days revealed a significant effect of Group, $F(1, 43) = 8.42$, $p < .01$, $\eta_p^2 = .16$, 95% CI [.01, .34], as well as a significant effect of Days, $F(4, 172) = 4.73$ $p < .01$, $\eta_p^2 = .1$, 95% CI [.01, .17]. The Days × Group interaction was significant, $F(4, 172) = 2.74$, $p < .05$, $\eta_p^2 = .06$, 95% CI [.00, .12]. Further analysis carried out to analyze the Group × Days interaction revealed that the groups significantly differed in Days 3, 4, and 5, smallest $t(43) = 2.76$. A repeated measures ANOVA carried out on the training days for the group Smooth revealed a significant effect of Days, $F(4, 84) = 2.55$, $p = .04$, $\eta_p^2 = .1$; pairwise comparisons showed that Day 1 differed from Day 2; there were no differences, however, between the Days 1, 3, 4, and 5. The same analysis carried out on the data from group Sand revealed a stronger effect of Days, $F(4, 88) = 5.82$, $p < .01$, $\eta_p^2 = .2$; pairwise comparisons showed that Days 1–3 significantly differed from Days 4–5. This pattern of results clearly revealed a long-term effect of the experience with the novel sandy context.

Figure 5B shows the group average locomotor activity (centimeters/minute) during the 1-hr test trial. A visual inspection suggests that, compared with the group Smooth for which the Sand context was new at the time of the test, the group Sand showed a significant reduced level of locomotor activity. This impression was confirmed by a one-way ANOVA showing a significant effect of Group, $F(1, 43) = 4.94$, $p < .05$, $\eta_p^2 = .10$, 95% CI [.00, .28].

The present results show evidence of habituation in the group given training in the Sand context. Animals given habituation training in the familiar, low salient smooth context showed less evidence of a decline in the locomotor activity during the habituation training, as evidenced by the Group × Day interaction during training. After a 24-hr interval from the last day of training, animals trained in the Sand context show lower levels of locomotor activity compared with the animals in the group Smooth for which the sandy context was new at the time of test. This is a clear example of long-term habituation in planarians.

Taking into account the housing conditions, we considered whether the relative novelty of the experimental context could be a relevant factor in the habituation of the exploratory or locomotor activity. The animals used in the present experiment came from a colony housed in a plastic container with a smooth surface; and were transferred for the duration of the experiment to plastic ice cube trays with a smooth surface. This would render the Sand context as more novel or salient than the Smooth one. We hypothesized, therefore, that changing the housing conditions should enable animals to become familiar with the sandy surface (both in the colony and the ice cube trays) and this should reduce the novelty of this context—and make the smooth context a salient one.[1] In other words, in Experiment 2A, planarians were housed in a smooth surface and displayed long-term habituation in the sandy surface. If the difference between housing and experimental surfaces is what enables the observation of long-term habituation, we should be able to replicate the results of Experiment 2A but reversing the housing and test environments. With all animals housed in a sandy surface, Group Smooth, trained, and tested in a novel smooth salient surface should show evidence of long-term

habituation; on the contrary, animals in the group Sand, trained with a familiar sandy context, should show little evidence of habituation during training and higher levels of activity when tested in the smooth context. Experiment 2B was designed to assess this hypothesis.

Experiment 2B

In Experiment 2B we changed the housing conditions: all animals were housed in a container with a sandy surface and transferred to ice cube trays with a sandy surface for the duration of the experiment. The animals were divided in two groups, Smooth and Sand. Following the same procedure described in Experiment 2A, animals in the Smooth group were given habituation training and test in the (relatively novel and salient) smooth context; animals in the Sand group were trained in the sandy context and subsequently tested in the novel salient smooth context.

Method.

Subjects and apparatus. The subjects were 32 brown planarians, which were assigned at random to one of two groups: Smooth ($n = 16$) or Sand ($n = 16$). The same apparatus and experimental contexts described for Experiment 2A were used. The animals were housed, however, under different conditions: Planarians were placed in a large sand tray for 25 days in order for them to familiarize with the sandy texture before the experiment started. They were then separated into ice cube trays that had a sandy surface (sand had been glued to the plastic ice cube trays using white silicone) 3 days before the start of the experiment. The animals remained housed on the sandy ice cube trays during the duration of the experiment. An unexpected effect of changing the housing conditions was that a higher than usual number of animals (13) died over the course of the experiment. This could be because of the hazardous nature of the sandy surface. Therefore, the analyses of the training and test sessions are restricted to 19 animals, nine in Group Smooth and 10 in Group Sand.

Procedure. The experiment replicates every aspect of the procedure described for Experiment 2A. The main manipulation was the housing conditions: the animals were housed in a sandy context to familiarize them with the Sand experimental context. During the first phase of the experiment, animals in the Smooth group were given habituation training during five daily sessions with the Smooth context whereas animals in the Sand group were exposed to the Sand context. The final test session was carried out in the Smooth context. We expected animals in the Smooth group, trained and tested in a relatively new smooth context, to show evidence of habituation (reduced locomotor activity) whereas animals in the group Sand (trained in a familiar context) would not show evidence of reduced locomotor activity.

Results and discussion. Figure 6A depicts the mean locomotor activity (centimeters/minute) of the animals in the experimental groups over the 5 days of habituation training. A mixed ANOVA with Group (Smooth vs. Sand) and Days revealed a significant effect of Days, $F(4, 68) = 2.57$ $p = .04$, $\eta_p^2 = .13$, 95% CI [.00, .24]. Neither the Group factor nor the Group × Days interaction was significant, maximum $F(1, 17) = 2.25$. This pattern of results suggests an effect of experience in both groups, revealed by a

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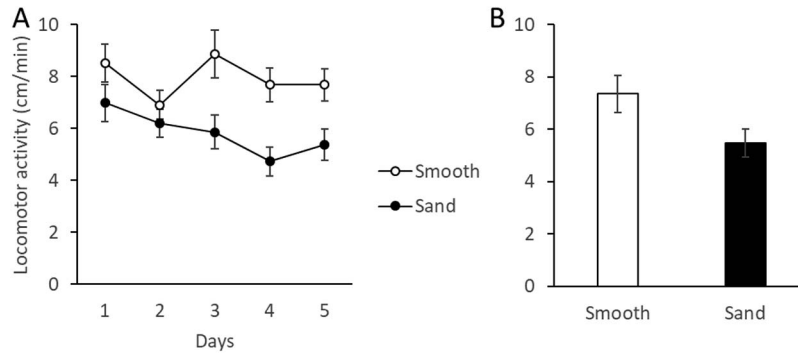[1] We thank Pam Birtill for suggesting this ingenious manipulation.

Figure 5. Experiment 2A results. (A) Group mean locomotor activity (±*SEM*) over the 5 days of the habituation training. (B) Group mean locomotor activity (±*SEM*) during the 1-hr test trial.

significant effect of the Days factor on the locomotor activity of the animals.

Figure 6B shows group average locomotor activity (centimeters/minute) during the 1-hr test trial. A visual inspection suggests that, compared with the group Sand for which the smooth context was relatively new at the time of the test, the group Smooth showed lower levels of locomotor activity. This impression was confirmed by a one-way ANOVA showing a significant effect of Group, $F(1, 17) = 5.24$, $p = .03$, $\eta_p^2 = .23$, 95% CI [.00, .49].

The results of Experiment 2B replicate those observed in Experiment 2A. When the animals were trained in a familiar surface (contextual cues) they failed to develop long-term habituation to the light stimulus presented on this context; however, when the animals were trained with a relatively novel context they developed long-term habituation.

General Discussion

The present research assessed habituation learning in planarians. Planarians possess a well-documented photophobic response sensitive to the intensity of the light (Davidson et al., 2011). The unconditioned photonegative response to the light has been assumed to habituate; however, this has hardly ever been put to test. Miller and Mahaffy (1930) showed that planarians' (*Cercaria amata*) locomotor activity increases when exposed to changes in luminance. The brighter the light, the greater the boost on spon-

taneous locomotor activity. They also showed that animals react to a sudden decrease in luminance (projection of a shadow) by increasing their activity; however, repeated shadow projections resulted in an inhibition of the swimming response—an instance of short-term habituation in response to a change in luminance levels.

The objective of Experiments 1 and 2 was to assess whether photonegative responses to the light stimulus would change with experience. Experiment 1 showed that planarians exposed to the light swam away from the light source; however, repeated exposures to the light resulted in longer times to leave the area adjacent to it and reduced the distance covered by the animals away from the light source over a period of 60 s. This reduction in the intensity of the photonegative response was shown to be an effect of habituation rather than mere fatigue or sensory adaptation to the light: when the animals were presented with an unexpected stimulus (dropped water in Experiment 1A; a 0.5-s shock in Experiment 1B) the photonegative response was restored—an instance of dishabituation.

Very little is known about the establishment of long-term habituation in the planaria. To the best of our knowledge, only one study (Westerman, 1963) showed evidence of habituation of the shrinking response to an intense light that was resistant to the passage of time (the reactivity to the light was diminished seven weeks after the habituation training). Unfortunately, Westerman did not assess the role of the contextual cues (or any other relevant
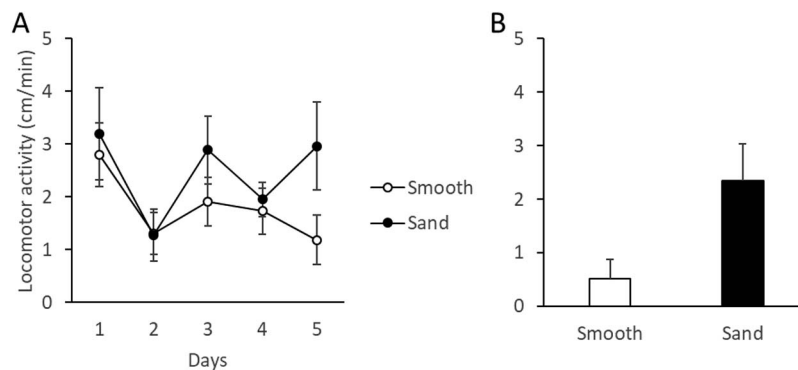

Figure 6. Experiment 2B results. (A) Group mean locomotor activity (±*SEM*) over the 5 days of the habituation training. (B) Group mean locomotor activity (±*SEM*) during the 1-hr test trial.

factor) in the reactivity to the light, so very little is known about the mechanisms underlying this instance of long-term habituation. In Experiment 2, we used a salient novel context to explore the habituation of the exploratory behavior shown by the animals.

In Experiment 2A, when the animals were exposed to a relatively familiar context (group Smooth, trained with a smooth surface similar to the one in the housing containers) they did not show any evidence of long-term habituation: there was no decrease in the levels of locomotor activity throughout sessions. On the contrary, when the animals were exposed to a novel context (with a sandy surface) they showed evidence of long-term habituation: the animals' levels of locomotor activity decreased significantly over the five habituation training sessions. Additionally, in a subsequent test carried out 24 hr after the last habituation training session, all animals were given an additional 1-hr exposure to the sandy context. Animals previously trained in the smooth context for whom the sandy context was new showed high levels of activity compared with the animals trained and tested in the presence of the sandy context. This pattern of results suggests that long-term habituation of the exploratory or locomotor activity is dependent upon the relative novelty of the context. The novelty of the context was defined by the presence of unfamiliar tactile cues: when the animals were trained in the presence of a surface with a smooth texture similar to the housing containers they did not show evidence of long-term habituation. However, when trained in the presence of a new texture (a sandy surface never experienced before) they showed evidence of long-term habituation. If this analysis is correct, changing the housing conditions to make the animals familiar with the sandy surface should allow us to replicate the results of Experiment 2A but reversing the housing and test environments. In Experiment 2B, the animals were housed in a sandy context for about 1 month before the onset of the experiment in which we used a smooth surface as the testing context. The animals given habituation training in the now familiar sandy context showed higher levels of activity in the smooth context during the test phase of the experiment relative to those given training and test in the relatively new smooth context. This seems to confirm that the novelty of the context is a key element in the development of long-term habituation of the exploratory or locomotor behavior.

The short-term habituation phenomena reported here (Experiments 1A and 1B) could be easily accommodated by a number of theories of habituation. When a stimulus is presented without being followed by any arousal-producing consequences, the natural responses to the stimulus are gradually attenuated. A number of authors have proposed that the attenuation of the unconditioned responses depend upon the existence of some sort of mental representation of the event. According to Sokolov (1963), for example, animals build up a neuronal model of the stimulus based on its sensory input. When the animal is exposed to a stimulus, in the absence of a match between the sensory input and an existent neuronal model the natural response elicited by the stimulus is displayed. When the sensory input matches an existent neuronal model, however, the response is inhibited. Through experience (accumulation of habituation training trials) an accurate model of the stimulus gradually develops weakening the response. Konorski (1967) proposed a similar approach and suggested that the natural response to a stimulus, the orienting response, was counteracted by the development of an inhibitory reflex when the stimulus was

experienced in the absence of other increasing arousal events (the *inhibitory perceptive recurrent reflex*; Konorski, 1967, p. 101). Presentation of an arousing event counters this inhibitory reflex and results in dishabituation.

Wagner's theory of short-term habituation (e.g., Wagner, 1976) shares in common with Sokolov's and Konorski's approaches the need for the existence of a representation of the stimulus to account for the gradually weakened unconditioned response. According to Wagner, recent presentations of the eliciting stimulus result in an active memory of these events in the STM that prevents its full processing and attenuates any responses it might elicit. In Experiments 1A and 1B the eliciting stimulus (i.e., presentation of the light stimulus) was repeatedly presented with short ITIs. The existence of an active mental representation of the light stimulus (self-generated priming) can account for the gradually reduced reactivity to the light. The presentation of a distractor (water drops or shock) would displace the mental representation of the light from the STM store; consequently, in subsequent presentations of the light its mental representation would not be active in the short-term information processor. In the absence of self-generated priming the stimulus is fully processed and any responses it commands displayed.

The advantage of Wagner's theory over other approaches is that, by reference to the same principles it can also account for the development of long-term habituation. When long intervals happen between the presentations of the eliciting events, external-generated priming can activate their mental representations. The source of external priming can be a stimulus consistently associated with the eliciting event (as in Pavlovian conditioning procedures), or the contextual cues in the standard habituation procedure. For long-term habituation to develop, therefore, an effective processing of the contextual cues is required to associate the context with the eliciting stimulus. The development of context-stimulus associations has been deemed unlikely in invertebrates, on the basis that their limited neural infrastructure would be insufficient to represent context information or associate or compress two biologically irrelevant stimuli (Lubow, 2010, p. 217). Contrary to the claims put forward by Lubow, we found in planarians strong sensitivity to changes in context processing, because it was a context manipulation what ultimately revealed long-term habituation.

Interpreting the long-term habituation reported in Experiment 2 might be slightly challenging. Wagner's theory could account for the results of Experiment 2 by assuming that the light underneath is independent of the tactile cues that provided the context. However, given that the light was presented throughout the training and testing sessions, it is likely that it also became part of the context.[2] At the moment, it is unclear if the animals sensed the light as part of the context or not. Contexts are usually defined as a set of diffuse (low intensity) stimuli that surround a particular learning experience (Bouton, 2010; Urcelay & Miller, 2014); in the present experiments, the light underneath could be said to provide aversive stimulation to the photophobic planarians. Accordingly, we can discuss the results of Experiment 2 in terms of Wagner's theory assuming that the surface was part of the stimulus context and the light an unconditioned or eliciting stimulus. In his seminal chapter Figure 7; Wagner, 1976), Wagner described an experiment con-

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[2] We thank an anonymous reviewer for this observation.

ducted in collaboration with Jesse W. (Bill) Whitlow and Penn L. Pfautz. In that experiment, exposure to the chamber (context) in which habituation training of the vasoconstriction response to a tonal stimulus took place, attenuated long-term habituation relative to a control group that did not receive additional exposure to the context. In our experiments, assuming the context and the light were associated during the training sessions, additional exposure to a similar surface as that of the context (in the ice cube trays) during the 23 hr that intervened between each habituation session should extinguish the association between the context and the light. In Wagner's theory, the context ought to be activated in A1 and the light representation ought to be activated in A2; thus, leading to an inhibitory association between these. Extinction of the context-light association in the ice cube trays would then prevent the observation of long-term habituation. Consistent with this, when the surface of the ice cube trays was dissimilar to that of habituation training, we successfully observed long-term habituation. This analysis is entirely consistent with the basic tenets of Wagner's theoretical approach. Wagner himself deemed the effect of context extinction interspersed with habituation training "a theoretically powerful prediction in that no other interpretation of habituation, including those which appeal to some associative mechanism seem prepared to anticipate" (Wagner, 1976, p. 121). These findings also replicate experiments in other invertebrate species described in the beginning of the article (Rankin, 2000; Tomsic et al., 1998).

As an alternative, we could assume that the light was perceived as part of the context rather than as a separate element. In that case, our experiments would be assessing the habituation of the exploratory behavior to a novel context. Notably, long-term habituation of the locomotor activity was only observed when the context was relatively new and not when the context contained familiar elements (i.e., the smooth surface shared by the home cages and the experimental dishes in Experiment 2A; and the sandy surface in Experiment 2B). This pattern seems to be consistent with the literature on distractor effects: although mere presentations to a target stimulus typically results in a diminution of the unconditioned response elicited by that stimulus, it is well established that if another stimulus is experienced before or after the target stimulus, habituation can be disrupted.

In a study by Robertson and Garrud (1983), rats were given one exposure trial to a particular flavor and the habituation of its neophobia—the natural response of rats to new flavor stimuli—was then assessed. Disruption of habituation was observed when the target flavor was followed by a dissimilar stimulus, sucrose followed by coffee, a postdistractor effect, but not when a similar distractor followed the target—lemon followed by coffee (Experiment 3; Robertson and Garrud provided independent evidence that the similarity between coffee and lemon was higher than the similarity between coffee and sucrose). When they reversed the order in a second experiment, the pattern of results was also reversed: disruption of habituation was observed when the target was preceded by a similar stimulus, coffee preceded by lemon, a predistractor effect, but not when a dissimilar distractor, sucrose, preceded coffee (Experiment 6). According to the authors of the study, the predistractor effect observed with similar stimuli is consistent with Wagner's theory of habituation: presentation of the distractor stimulus (lemon) would activate this stimulus in A2. Immediate presentation of a similar target stimulus (coffee), shar-

ing common features with the distractor stimulus, would fail to activate the common features in A1 in the STM unit, limiting the processing of the coffee stimulus and hence the habituation of the neophobic unconditioned response (for a discussion of distractor effects on habituation of complex stimuli see Artigas, Sansa, & Prados, 2012). In the present Experiment 2, the home context could be working as a predistractor stimulus limiting the processing of a significant proportion of the features of the target experimental context. Only when the experimental context is distinctive from the home context, adequate processing of its features would be guaranteed, resulting in the establishment of an accurate representation in short- and long-term memory. This would allow for the effective long-term habituation observed in Experiment 2.

The experiments presented here have a number of implications for a comparative analysis of habituation, and in light of Wagner's theoretical approach, shed light on the basic information processes underlying habituation in an invertebrate species. Experiment 1 suggested that short-term habituation in planarians follows similar principles to those reported in vertebrates. The findings of Experiment 2 replicate the only demonstration of long-term habituation that we are aware of (Westerman, 1963), but in addition show that this effect can be sensitive to manipulations of the context. These results are consistent with predictions of Wagner's theory, and bolster the claim that invertebrates are sensitive to contextual manipulations, unlike what has been proposed by Lubow (2010).

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