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Rozprawa doktorska oparta na cyklu publikacji

# Nowość i zmiany w złożoności środowiska a zachowanie się zwierząt - badania w nurcie porównawczej psychologii zwierząt

Rozprawa doktorska napisana pod kierunkiem
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**Abstrakt**

Przedstawiona rozprawa doktorska podejmuje temat roli nowości stymulacji oraz zmian w złożoności w regulacji zachowania się zwierząt. W badaniach nad zachowaniem zwierząt związanych z oddziaływaniem na nie środowiska kluczową rolę pełnią reakcja na nowość i zachowania eksploracyjne. Złożoność środowiska, reakcja na nowość i zachowania eksploracyjne pozostają ze sobą ściśle powiązane.

Rozprawa opiera się na cyklu pięciu artykułów, a wyniki badań zostały przeanalizowane pod kątem występowania aktywności eksploracyjnej i reakcji na nowość. Cztery badania zostały przeprowadzone na szczurach, a jedno na żółwiach lądowych.

W badaniach wprowadzano różne rodzaje nowości: ruchome elementy, dodatkowe elementy, bodźce świetlne, zupełnie nowe obiekty. Wyniki pokazały pewne wspólne właściwości zachowania oraz pewne różnice zależne od rodzaju zastosowanej manipulacji. We wszystkich badaniach z udziałem szczurów wzrost złożoności środowiska i zastosowane nowe bodźce wpłynęły na wzrost aktywności eksploracyjnej. W badaniu z udziałem żółwi lądowych zaobserwowano reakcję na nowość związaną z prezentowaniem zwierzętom nowych elementów.

**Abstrakt w języku angielskim**

The dissertation presented here explores the role of the novelty of stimulation and changes in complexity in regulating animal behavior. In the studies of animal behavior related to environmental influences, response to novelty and exploratory behavior both play a crucial role. Environmental complexity, response to novelty, and exploratory behavior remain closely linked.

The dissertation is based on a series of five articles, and the results were analyzed in terms of the occurrence of exploratory activity and response to novelty. Four studies were conducted on rats and one on land tortoises.

In the presented studies, different types of novelty were introduced: moving items, additional items, light stimuli, and completely new objects. The results showed some common behavioral characteristics and differences depending on the type of manipulation used. In all studies involving rats, the increased complexity of the environment and the novel stimuli increased exploratory activity. In the study involving land tortoises, a response to novelty associated with the presentation of new items to the animals was observed.

# Spis treści

# 1. Wprowadzenie

Celem badań przedstawionych w niniejszej pracy jest analiza związku nowości stymulacji oraz zmian złożoności środowiska i zachowania się zwierząt. W szczególności, prezentowane badania zawierają w sobie manipulacje eksperymentalne, które poprzez zmianę złożoności, konfrontują badane zwierzęta z nowością wyrażającą się zwiększeniem lub zmniejszeniem złożoności środowiska testowego. Wszystkie organizmy, od najprostszych po najbardziej złożone stają w obliczu wyzwań generowanych przez środowisko i muszą sobie z tymi wyzwaniami radzić. Sposób w jaki organizm poradzi sobie z bodźcami środowiskowymi ma kluczowe znaczenie dla jego przetrwania. Wyniki przedstawionych badań zostały omówione pod kątem regulacji zachowań eksploracyjnych. Podobnie analizowana była reakcja na nowość, tzn. wzięto pod uwagę rodzaje nowości oraz rodzaje odpowiedzi na nowe bodźce manifestujące się w zachowaniu zwierząt.

## 1.1 Złożoność środowiska

Złożoność jest jednym z ważnych aspektów środowiska (Osborn i Hunt, 1972). Wzorce złożoności środowiska różnią się w zależności od gatunku, jednak zwykle można wyróżnić kilka wspólnych parametrów takich jak przestrzeń, zagęszczenie społeczne czy nowość oferowana w środowisku (Lewis, 2004). Złożoność może być definiowana na różne sposoby: jako liczba dostępnych w środowisku elementów, jako liczba dostępnych źródeł stymulacji czy też jako relacje zachodzące między występującymi w środowisku obiektami (Pervin, 1978). W rozważaniach na temat złożoności środowiska przydatnym terminem mogą być również afordancje - termin wprowadzony w latach siedemdziesiątych przez J.J. Gibsona (Gibson, 1979/2014). Afordancje mogą być rozumiane jako to co dane środowisko oferuje jednostce i możliwe formy zachowania wynikające z dostępności tych ofert (Rietveld i Kiverstein, 2014).

Badanie wpływu wzbogaconego środowiska na funkcjonowanie mózgu zwierząt laboratoryjnych poprzez dostarczanie im źródeł stymulacji takich jak nowe obiekty, inne zwierzęta czy większa przestrzeń ma już dość długą historię (Lewis, 2004). Już w latach czterdziestych XX wieku Donald Hebb w ramach eksperymentu pozwolił szczurom laboratoryjnym przemieszczać się swobodnie po swoim domu. Okazało się, że doświadczenie przebywania w bardziej złożonym środowisku, w porównaniu do klatek laboratoryjnych, zwiększyło u szczurów zdolność uczenia się i lepiej radziły sobie one potem w pokonywaniu labiryntu (Hebb, 1949). Badania pokazują, że złożoność środowiska jednocześnie redukuje zachowania lękowe i zwiększa aktywność zwierzęcia (Benaroya - Milshtein, 2004).

Wzbogacenie środowiska najczęściej kojarzy się z umieszczeniem w przestrzeni elementów, które dadzą zwierzętom możliwość eksploracji. Tymczasem warto zwrócić uwagę na fakt, że już samo zwiększenie wielkości klatki może istotnie wpłynąć na aktywność zwierząt (Manosevitz i Pryror, 1975). Jak podają autorzy, środowisko, w którym jest odpowiednio dużo przestrzeni do biegania i eksplorowania jest bardziej stymulujące niż standardowe klatki stosowane w laboratoriach.

Warto zaznaczyć, że badania poświęcone wpływowi warunków środowiska na zachowanie i zdolności poznawcze zwierząt nie tylko służą badaczom jako źródło wiedzy, ale także przyczyniają się do poprawy warunków bytowania samych zwierząt w laboratoriach, ogrodach zoologicznych i wielu innych miejscach. Nie tylko szczury były przedmiotem badań związanych ze wzbogaceniem i złożonością środowiska. Badano i nadal bada się inne gatunki ssaków takie jak koty czy małpy oraz gatunki należące do innych gromad kręgowców (Rosenzweig i Bennet, 1972).

W rozważaniach na temat złożoności środowiska nie można pominąć zaproponowanej przez Godfreya-Smitha (1998) teorii o złożoności środowiska (*Environmental Complexity Thesis - ECT*). ECT jest traktowana przez autora jako ogólna zasada ewolucji poznania, która oznacza, że funkcją poznania jest umożliwienie organizmowi radzenia sobie ze złożonością środowiska. Godfrey-Smith (2002) podaje również, że najlepsza definicja tego czym jest złożoność jest prosta - złożoność to heterogeniczność czyli różnorodność i urozmaicenie środowiska oraz możliwość podejmowania różnych działań w środowisku w zależność od tego co ono oferuje.

W odpowiedzi na zmiany w środowisku, zwierzęta dostosowują swoje zachowanie, zależnie od stopnia rozwoju. Plastyczność zachowania jest związana ze złożonością budowy i funkcji układu nerwowego. Chcąc prześledzić procesy związane z ewolucją zachowania w zależności od środowiska należy skupić się na dwóch aspektach: wrodzonych wzorcach zachowań oraz na uczeniu się (Mery i Burns, 2010). Jak podają Berrigan i Scheiner (2004) by plastyczność zachowania ewoluowała niezbędne są następujące czynniki: różnorodność środowiska, wiarygodność wskazówek, korzyści, które będą przewyższać koszty modyfikacji zachowania oraz genetyczne uwarunkowania, które pozwolą by plastyczność mogła wystąpić.

Wrodzone wzorce zachowania kształtują się z pokolenia na pokolenie, natomiast uczenie się jest procesem zachodzącym indywidualnie dla każdej jednostki. Różnorodność środowiska odgrywa kluczową rolę w tym aby oba te procesy mogły wystąpić. Wrodzone wzorce dają zwierzęciu korzyści w momencie kiedy środowisko, w którym przebywa jest

stosunkowo stabilne, a zmiany zachodzą dość wolno. Uczenie się z kolei pomaga przetrwać zwierzęciu wtedy kiedy zmiany zachodzą w szybszym tempie (Stephens, 1991).

## 1.2. Zachowania eksploracyjne

Zachowania eksploracyjne są specyficzną formą zachowania. Nie są powodowane potrzebą zaspokojenia podstawowych potrzeb życiowych takich jak zaspokojenie głodu czy popędu seksualnego (Pisula i Osiński, 1996). Trudno jest je zdefiniować w jednoznaczny sposób, można jednak przyjąć, że zachowania te występują wtedy, kiedy zwierzę podejmuje działanie mające na celu zebranie informacji o otaczającym je środowisku (Mc Reynolds, 1962). Autorzy zajmujący się problematyką tego rodzaju zachowań przedstawiają różne koncepcje dotyczące tego jak można je różnicować. Mc Reynolds postuluje istnienie przynajmniej trzech rodzajów zachowań eksploracyjnych. Pierwszym z nich jest zachowanie przystosowawcze do nowości *(ang. novelty - adjustive behavior)* polegające na tym, że zwierzę nie ze swojej inicjatywy zostaje skonfrontowane z nowością pojawiającą się w otoczeniu i musi się w jakiś sposób dostosować do nowej sytuacji. Aby zredukować napięcie spowodowane pojawieniem się nieznanego bodźca zwierzę eksploruje nowy obiekt, w wyniku czego coś "nieznanego" staje się czymś "znanym". Co istotne, w tym przypadku stopień nowości nie może być zbyt duży by zwierzę nie wycofało się z aktywności eksploracyjnej. Drugim rodzajem zachowania eksploracyjnego wymienionym przez autora jest poszukiwanie nowości *(ang. novelty - seeking behavior)*. Tutaj, w przeciwieństwie do pierwszego rodzaju, zwierzę podejmuje z własnej inicjatywy aktywność polegającą na poszukiwaniu nowości. Trzecim rodzajem zachowania eksploracyjnego jest poszukiwanie nowości zorientowane na cel *(ang. goal - oriented novelty seeking behavior)*. W tym przypadku poszukiwanie nowości można traktować jako środek do celu, na przykład kiedy zwierzę przemierza i eksploruje nowy obszar w celu znalezienia pożywienia. Rodzaj pożywienia może być jak najbardziej znany zwierzęciu wcześniej, czyli cel nie jest nowy. Nowa natomiast może być droga (konfrontacja z nowymi bodźcami), którą zwierzę musi przemierzyć, by ten znany cel osiągnąć.

Jeśli chodzi o różne rodzaje zachowań eksploracyjnych, warto wspomnieć również o rozróżnieniu dokonanym przez Welkera (1957), który zaproponował podział na zachowania o charakterze dobrowolnym oraz wymuszonym. Eksploracja dobrowolna ma miejsce wtedy kiedy zwierzę ma możliwość wyboru czy chce się skonfrontować z danym bodźcem czy nie. W warunkach laboratoryjnych można to uzyskać poprzez umożliwienie zwierzęciu

swobodnego dostępu do areny doświadczalnej, jednocześnie pozostawiając mu możliwość schowania się na przykład w transporterze. Eksploracja wymuszona ma miejsce wtedy, kiedy zwierzę nie ma możliwości wyboru i jest zmuszone do konfrontacji z nowością. Można założyć, że istnieją czynniki wyzwalające zachowania eksploracyjne. Berlyne (1962) jako takie czynniki wymienia nowość, złożoność, zmianę oraz zaskoczenie (*surprisigness*). Ten sam autor (Berlyne, 1960) zaproponował podział zachowań eksploracyjnych na dwa rodzaje. Pierwszym z nich jest *diversive exploration,* którego funkcją jest zbieranie ogólnych informacji o otaczającym środowisku. Zaś drugi rodzaj to *specific exploration,* które polega na eksploracji ukierunkowanej na konkretne nowe obiekty.

Szczury i inne gryzonie stanowią główny obiekt badań nad zachowaniami eksploracyjnymi. Warto jednak zaznaczyć, że wśród innych zwierząt zaliczanych do gromad kręgowców również takowe zachowania występują. Warto tutaj wspomnieć o tym, że tradycyjny podział na kręgowce wyższe i niższe często jest poddawany krytyce. Być może jednak nie jest on zupełnie nieuzasadniony. Patrząc z perspektywy ewolucji mózgu, w literaturze można spotkać określenie "mózg owodniowców" (*ang. amniote brain*). Określenie to dotyczy gadów, ptaków i ssaków, czyli kręgowców, u których rozwój zarodkowy występuje na lądzie. Wygląda na to, że u owodniowców można spotkać podobne mechanizmy dotyczące działania mózgu. Związane jest to z ewolucyjną tendencją do kortykalizacji mózgu wymienionych wcześniej gromad zwierząt (Pisula, 2020).

Analizując temat zachowań eksploracyjnych warto zastanowić się nad adaptacyjną funkcją tych zachowań. Ten aspekt można rozważać w ujęciu zysków i strat dla zwierzęcia w związku z podjęciem eksploracji. Do zysków można zaliczyć pozyskiwanie informacji z otoczenia, na przykład na temat pożywienia czy dogodnego miejsca schronienia, do kosztów można zaliczyć narażenie się na atak drapieżnika czy zranienie w wyniku wypadku (Pisula, 2003). Tolman (1948) wskazuje, że zachowania eksploracyjne mogą przyczynić się do budowania mapy przestrzennej środowiska.

W 1958 M. Glenzer dokonał podsumowania wyników badań nad zachowaniami eksploracyjnymi, z którego wyłania się obraz pewnych prawidłowości dotyczących tych zachowań. Na potrzeby niniejszej pracy zostaną wymienione niektóre z nich: miejsce gdzie występują bodźce złożone jest bardziej eksplorowane przez szczury, niż miejsca gdzie złożoność nie występuje, intensywność eksploracji spada wraz z doświadczaniem danej sytuacji, możliwość podjęcia aktywności eksploracyjnej ma wartość nagradzającą, spadek aktywności eksploracyjnej w znanej sytuacji doświadczalnej można uogólnić na sytuacje podobne.

Badanie mechanizmów motywacyjnych zachowań eksploracyjnych nastręcza pewnych trudności. Trudności te związane są w dużej mierze z nieprecyzyjnością definicji tychże zachowań. Każde zachowanie pozostaje w związku ze stymulacją płynąca ze środowiska, jednak należy zwrócić uwagę na to, co decyduje w danym momencie o wystąpieniu eksploracji, czy jest ona motywowana wewnętrznie czy zewnętrznie. W badaniach laboratoryjnych często stosuje się testy badające eksplorację wymuszoną, tymczasem duże nadzieje wiąże się z eksperymentami badającymi eksplorację spontaniczną (Pisula, 2020).

## 1.3. Reakcja na nowość

W badaniach na zwierzętach, związanych z oddziaływaniem na nie środowiska kluczową rolę pełnią reakcja na nowość i zachowania eksploracyjne. Zjawiska takie jak złożoność środowiska, zachowania eksploracyjne i reakcja na nowość pozostają ze sobą ściśle powiązane (Berlyne, 1978). Nowość można zdefiniować jako odchylenie od oczekiwanego prawdopodobieństwa wystąpienia jakiegoś zdarzenia na podstawie wcześniejszych informacji jakie posiada organizm i wewnętrznych szacunków prawdopodobieństwa warunkowego (Berns i in., 1997). Nowość można również traktować jako źródło informacji. Organizmy regulują swoje zachowanie w oparciu o informacje płynące z otoczenia (Modlińska i Pisula, 2020). Wśród kręgowców reakcję na nowość obserwuje się głównie u ptaków i ssaków. Badania pokazują jednak, że reakcja na nowość i potrzeba eksploracji nowych obiektów jest obecna również u niektórych gadów, w tym żółwi (Greenberg, 2003).

Berlyne (1978) zwraca uwagę na istotną kwestię dotyczącą tego, że określenie, że coś jest “nowe” może mieć kilka znaczeń. Można zatem mówić o nowości krótkoterminowej, co oznacza, że zmiana będąca nowością pojawia się w ciągu kilku minut w stosunku do uprzedniego stanu jaki zastał organizm. Nowość krótkoterminowa różni się od nowości długoterminowej, którą rozpatruje się w stosunku do wszystkiego, co było wcześniej doświadczane. Kolejne aspekty związane ze zjawiskiem doświadczania nowości to zaskoczenie, czyli sytuacja, w której to, co spodziewane różni się od tego, co się pojawia oraz nieścisłość czyli pojawienie się w środowisku elementów nowości, które nie powinny wystąpić razem (Berlyne, 1978). Reakcja na nowość jest związana z wcześniejszymi doświadczeniami organizmu (Barto i in., 2013). Berlyne (1960) dokonał rozróżnienia między nowością absolutną i względną. Z pierwszą z nich organizm ma do czynienia wtedy, kiedy

nigdy wcześniej nie doświadczał żadnej z cech nowego bodźca. Natomiast o nowości względnej można mówić wtedy, kiedy pewne cechy bodźca były wcześniej znane zwierzęciu, ale w danej sytuacji występują one w nieznanym wcześniej układzie czy kombinacji.

Z reakcją na nowość związane są dwa zjawiska: neofobia i neofilia. Pierwsze z nich oznacza lęk przed nowymi bodźcami i nowymi sytuacjami (Grennberg i Mettke Hofmann, 2001). Reakcje neofobiczne występują u wszystkich gatunków ssaków i ptaków (Corey, 1978). Neofilia to dążenie do nowości pojawiającej się w otoczeniu (Greenberg, 2003). Neofobia pełni funkcję chroniącą zwierzę przez niebezpieczeństwem, zaś neofilia związana jest z pozyskiwaniem przez zwierzę informacji z otoczenia (Greenberg i Mettke - Hofmann, 2001).

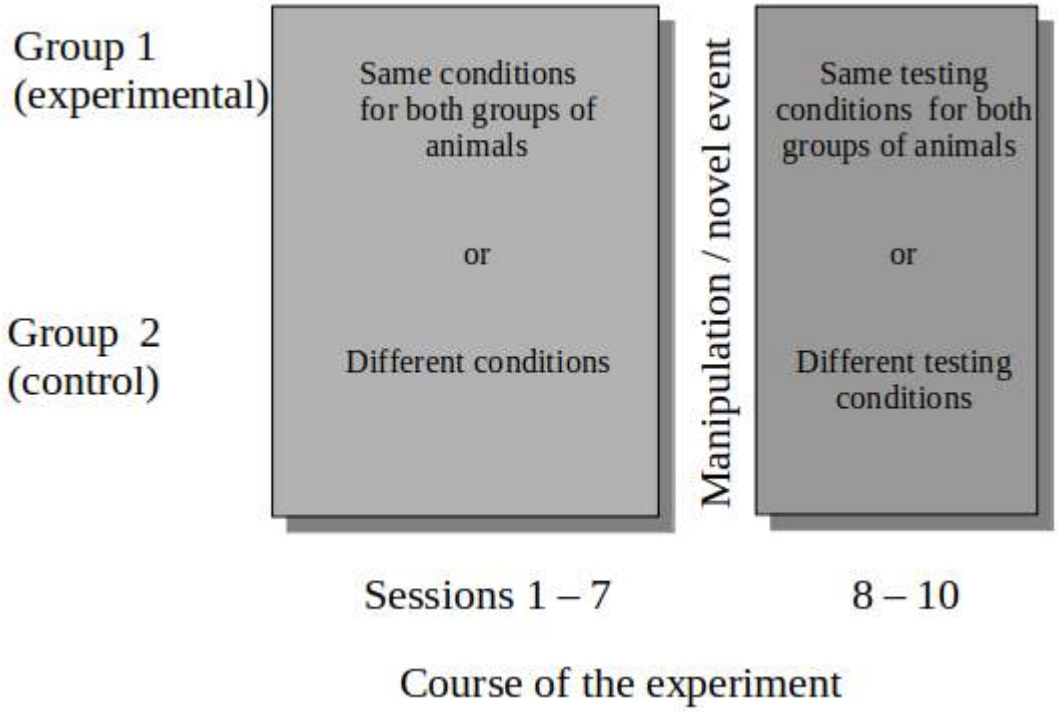
Zwierzęta mogą regulować poziom nowości pojawiającej się w otoczeniu poprzez własną aktywność. Mogą się zbliżać do źródła nowości lub wycofywać. Dlatego też można przyjąć, że w środowisku naturalnym jest mało prawdopodobne by organizm miał do czynienia z całkowicie nową stymulacją (Modlińska i Pisula, 2020). By nowość miała charakter nowości absolutnej (Berlyne, 1960) organizm nie mógłby mieć żadnych oczekiwań związanych z wcześniejszym doświadczeniem. Taka sytuacja musiałaby raczej zostać wytworzona sztucznie, celowo bądź nie, przez człowieka (Modlińska i Pisula, 2020).

## 2. Różne aspekty złożoności środowiska - cykl badań

Na niniejszą pracę składa się cykl pięciu badań dotyczących różnych aspektów złożoności środowiska, reakcji na nowość i ich wpływu na aktywność eksploracyjną zwierząt. Cztery z nich zostały przeprowadzone na szczurach, jedno na żółwiach lądowych (*Testudo hermanni* oraz *Agrionemys horsfieldii*). We wszystkich badaniach na szczurach wykorzystano tę samą aparaturę badawczą i prowadzono badanie według tego samego protokołu (Pisula i Modlińska, 2020), zastosowano jednak różne rodzaje manipulacji jako nowość pojawiającą się w środowisku. Manipulowano złożonością przestrzenną obiektów, ich ilością, mobilnością oraz zastosowano bodźce świetlne. Z kolei w badaniu na żółwiach lądowych wykorzystano bodźce wizualne.

Warto zwrócić uwagę na fakt, że jednym ze znaczących problemów dotyczących badań na kręgowcach jest kontrola poziomu stresu u badanych zwierząt (Langkilde i Shine, 2006). Duży poziom stresu u badanych zwierząt może wprowadzać czynniki zakłócające (np. pojawienie się stereotypii behawioralnych), które utrudniają uzyskanie odpowiedzi na pytania dotyczące problemu badawczego (Belliure i in., 2004; Gillette i in., 1995). Wszystkie badania

przedstawione w niniejszym cyklu zostały przeprowadzone w warunkach nisko stresowych. W badaniach z udziałem szczurów zastosowano ten sam protokół badawczy który obejmował siedem dni fazy habituacyjnej oraz trzy dni testowe. W fazie habituacyjnej stosowano zawsze to samo ustawienie tuneli, a następnie w fazie testowej wprowadzano zmiany wg. ogólnego schematu - Rycina 1.




Rycina 1. Schemat wprowadzania zmian w badaniach z udziałem szczurów.

## 2.1. Wpływ zmienności wzbogaconego środowiska domowego na eksplorację u szczurów.

Modlinska, K., **Chrzanowska, A.**, & Pisula, W. (2019). The impact of changeability of enriched environment on exploration in rats. *Behavioural Processes*, *164*, 78-85.

Celem badania było znalezienie odpowiedzi na pytanie jaki wpływ na reakcję na nowość będą miały warunki życia w środowiskach o różnym stopniu złożoności oraz jej zmienności. Doświadczenie zmienności środowiska lub jej braku może mieć wpływ na reakcję na nowości u zwierzęcia. Możliwość doświadczenia różnych poziomów złożoności może odgrywać ważną rolę w adaptacji do nowości. Warto zaznaczyć, że zmiana zachowania w badaniach gdzie wprowadza się nowe elementy może być w jakimś stopniu

odzwierciedleniem zmiany preferencji dotyczącej samej złożoności, a nie odpowiedzią na nowość (Pisula i in., 2006). W kontrolowanych warunkach laboratoryjnych jest możliwość zaaranżowania środowiska wzbogaconego, które dostarczy zwierzętom stymulacji poznawczej, która pozwoli na wystąpienie zachowań eksploracyjnych (Baroncelli i in., 2010). Najczęściej jednak, niezależnie od jakości wprowadzonego wzbogacenia, środowisko życia zwierząt laboratoryjnych charakteryzuje się stałością aranżacji przestrzeni i obecnych w niej elementów. Stosunkowo niewiele badań podejmuje temat wpływu zmienności środowiskowej w warunkach laboratoryjnych. Przy czym trzeba zaznaczyć, że zmienność środowiska nie musi oznaczać wzrostu złożoności środowiska. Zmienność można uzyskać na przykład poprzez zmianę ustawienia elementów, które są obecne w środowisku, nie zmienia to jednak jego złożoności. W warunkach naturalnych pewne cechy środowiska mogą być przewidywalne (cykl dzień/noc), a pewne nieprzewidywalne (pojawienie się drapieżników, zmiany pogody) - (Wingfield, 2008). Zmienność środowiska może mieć znaczący wpływ na zachowanie zwierząt.

By móc sprawdzić jak doświadczenia związane ze złożonością i zmiennością środowiska wpływają na przyszłe radzenie sobie w sytuacji, kiedy pojawia się nowość w otoczeniu, szczury podzielono na trzy grupy. Dwie z nich przebywały przez okres trzech miesięcy w kojcach wyposażonych w różne elementy pozwalające szczurom eksplorować i manifestować typowe dla gatunku zachowania. W jednej grupie elementy wzbogacenia pozostawały cały czas w takim samym układzie, natomiast w drugiej ułożenie obiektów było codziennie zmieniane. Trzecią grupę stanowiła grupa kontrolna, która przebywała w standardowych klatkach laboratoryjnych. Po trzech miesiącach przeprowadzono badanie polegające na obserwacji zachowań eksploracyjnych i reakcji na nowość. Badanie było przeprowadzone w aparaturze składającej się z trzech komór. Komora centralna prowadziła do dwóch komór, gdzie umieszczono tunele. W jednej komorze ustawienie tuneli było niezmienne przez całą fazę habituacji i fazę testową. W drugiej zaś po fazie habituacyjnej zwiększono liczbę tuneli.

Wyniki badania pokazały z jednej strony, że na początku fazy testowej, szczury trzymane w standardowych warunkach przejawiały większą aktywność eksploracyjną w strefach, gdzie znajdowały się tunele, a mniej czasu spędzały w strefach gdzie obiektów nie było. Być może to, że przebywały one wcześniej w warunkach, które nie dostarczyły im wystarczającego poziomu stymulacji, spowodowało zwiększone zapotrzebowanie na interakcję z nowym środowiskiem. Z drugiej strony, we wszystkich grupach pod koniec fazy habituacyjnej odnotowano zbliżony poziom aktywności, co może wskazywać na zbliżony

poziom habituacji do środowiska eksperymentalnego. W fazie testowej, kiedy wprowadzono elementy nowości w postaci zwiększenia liczby tuneli w jednej ze stref, u wszystkich badanych grup nastąpiło wzmożenie zachowań eksploracyjnych w stosunku do tychże obiektów. Jednak u szczurów z grup utrzymywanych przed testem w środowisku wzbogaconym w kolejnych dniach testowych spadał poziom zainteresowania nowymi elementami. Natomiast szczury z grupy kontrolnej wykazywały wysoki poziom eksploracji w stosunku do nowych obiektów w kolejnych dniach testowych. Utrzymujący się wysoki poziom eksploracji we wszystkich dniach testowych w grupie kontrolnej może świadczyć o tym, że u tych szczurów tempo adaptacji do zmiany było wolniejsze niż w pozostałych grupach. Warto zaznaczyć, że obiekty użyte w badaniu stanowiły różne rodzaje nowości (Berlyne, 1960) w zależności od badanej grupy. U szczurów z grup ze wzbogaceniem środowiska nowość ta miała charakter nowości względnej. Przed badaniem miały one styczność z tunelami bardzo podobnymi do tych, które zostały użyte w badaniu. Dla nich nowością nie były więc same tunele, tylko ich organizacja przestrzenna użyta w manipulacji eksperymentalnej. Natomiast szczury z grupy kontrolnej pierwszy raz z tego typu obiektami zetknęły się dopiero w aparaturze badawczej, dlatego dla nich nowość ta miała charakter nowości absolutnej.

Różnice w aktywności eksploracyjnej zaobserwowano również między grupami ze środowisk wzbogaconych. U szczurów hodowanych w stabilnym środowisku nastąpił spadek eksploracji obiektów w strefie niezmienionej po tym jak wprowadzono zmianę w drugiej strefie. Natomiast u szczurów hodowanych w środowisku zmiennym nie odnotowano różnic w eksplorowaniu obiektów w strefie niezmienionej. W obu grupach jednak nie miało to wpływu na wzrost ilości czasu poświęconego na eksplorację nowych obiektów. Warto też zaznaczyć, że w grupie utrzymywanej przed testem w środowisku wzbogaconym zmiany wprowadzane w zagrodzie mieszkalnej dotyczyły tylko ustawienia obiektów w przestrzeni. Nie pojawiały się żadne nowe obiekty. Nowość pojawiająca się w tej grupie miała charakter zaskoczenia, posługując się określeniem przytoczonym przez Berlyne'a (1978). Oznacza to, że obiekty były ustawione inaczej, niż można się było spodziewać, zważywszy na uprzednie doświadczenie.

Brak różnic między grupami hodowanymi w zmiennym i niezmiennym środowisku wzbogaconym, wobec stwierdzonych różnic pomiędzy grupami pochodzącymi ze środowiska wzbogaconego oraz grupą kontrolną mogą oznaczać, że samo wzbogacenie środowiska, nawet jeśli nie zachodzą w nim zmiany, jest wystarczająco stymulujące i umożliwia szczurom samodzielne manipulowanie poziomem złożoności poprzez interakcję z obiektami,

a co za tym idzie, pozwala regulować poziom stymulacji pochodzącej z owego środowiska. Wydaje się iż zmiany, których źródłem nie jest aktywność własna zwierzęcia, o ile nie powodują całościowej reorganizacji dostępnej przestrzeni nie odgrywają kluczowej roli w regulacji zachowania się zwierząt.

## 2.2. Reakcja szczura na nowość i wzrost złożoności środowiska wynikający z wprowadzenia obiektów ruchomych vs. nieruchomych w teście swobodnej eksploracji.

**Chrzanowska, A**., Modlinska, K., Goncikowska, K., & Pisula, W. (2022). Rat's response to a novelty and increased complexity of the environment resulting from the introduction of movable vs. stationary objects in the free exploration test. *Plos One, 17*(12), e0279006.

W tym badaniu manipulacja eksperymentalna polegała na wprowadzeniu dwóch zmian. Pierwszą z nich była również zmiana przestrzenna polegająca na dodaniu tunelu, jednak drugim nowym elementem była zmiana dotychczasowych właściwości znanych wcześniej obiektów.

Środowisko wysoko złożone to w warunkach naturalnych często środowisko nieprzewidywalne. Nieprzewidywalność związana jest z trudną do przewidzenia zmiennością środowiska. Zmiany mogą dotyczyć wielu aspektów (na przykład pojawienie się nowych obiektów, pojawienie się drapieżników, ingerencja ze strony człowieka). Z nieprzewidywalnością wiąże się stan psychologiczny określany jako niepewność, który pojawiać się może w sytuacjach, kiedy organizm zostaje skonfrontowany ze zdarzeniami zachodzącymi w otoczeniu, których konsekwencji nie może przewidzieć (Inglis, 2000). Organizmy o różnej złożoności biologicznej muszą się mierzyć z niepewnością i tym, jakie konsekwencje mogą się pojawiać w związku z napotkaniem nowych bodźców w środowisku (Kahneman i Tversky, 1982). Można założyć, że zastosowana w badaniu manipulacja będzie spełniać wymienione za Berlyne'm (1962) w podrozdziale o zachowaniach eksploracyjnych czynniki inicjujące zachowania eksploracyjne takie jakie: nowość, złożoność, zmiana i zaskoczenie.

W badaniu wykorzystano trzy grupy szczurów. Wszystkie w fazie habituacyjnej miały do czynienia z tym samym ustawieniem elementów. Następnie w fazie testowej w pierwszej grupie dokonano zmiany właściwości elementów z nieruchomych na ruchome.

Była to więc manipulacja polegająca na zwiększeniu zmienności środowiska. W drugiej grupie dokonano dwóch rodzajów zmian, po pierwsze zwiększono liczbę tuneli, a po drugie jeden z nich był ruchomy, zwiększono więc zarówno złożoność jak i zmienność środowiska testowego. Natomiast w trzeciej grupie zastosowano manipulację przestrzenną polegająca na dodaniu tuneli nieruchomych, co powodowało zwiększenie złożoności, a nie zmienności środowiska testowego (oczywiście z wyłączeniem jednorazowej zmiany związanej z samą manipulacją).

Wyniki badania pokazały, że u szczurów z grupy gdzie zamieniono dwa nieruchome tunele na dwa ruchome nie wykazano różnic w zachowaniu. Można więc wnioskować, że sama zmiana właściwości znanych wcześniej przedmiotów nie była wystarczająco stymulująca. Jednak trzeba tu zwrócić uwagę na bardzo istotną kwestię. Mianowicie w fazie testowej zwierzęta zastały elementy, które w pierwszym zetknięciu się z nimi nie różniły się niczym w stosunku do tych, które znały już z fazy habituacyjnej. Możliwa interpretacja tego wyniku jest związana z teorią mówiącą o formułowaniu oczekiwań w stosunku do środowiska na podstawie uprzednich doświadczeń (Kahneman i Tversky, 1982). Żeby wykryć zmianę i doświadczyć tego, że znane obiekty posiadają nowe właściwości zwierzęta musiały wejść na nie i tym samym wprawić je w ruch. Być może wykrycie tej zmiany było hamowane właśnie przez brak dostatecznej motywacji do eksploracji związany z zastaniem pozornie niezmiennego środowiska. Szczury z tej grupy wchodziły prędzej czy później w interakcje z ruchomymi tunelami, jednak nie odnotowano istotnych różnic w ich zachowaniu względem aktywności w fazie habituacji. Zatem miały zdolność do wykrycia zmiany, być może jednak nowość w postaci ruchomych tuneli nie była wystarczająco znacząca by wywołać istotne zmiany w zachowaniu. Szczury z dwóch pozostałych grup, czyli grupy gdzie zwiększono liczbę tuneli i dodano tunel ruchomy oraz z grupy gdzie zwiększono liczbę tuneli, spędzały istotnie więcej czasu w strefach, w których wprowadzono zmianę. Zwierzęta z obu grup przejawiały zachowania takie jak obwąchiwanie, wspinanie się na tunele czy dotykanie ich, co można określić za Berlyne'm (1960) jako *specific exploration.* Co więcej, jeśli chodzi o określenie typu nowości można tutaj zastosować termin również zaproponowany przez tego samego autora (Berlyne, 1960) i określić ten rodzaj nowości jako nowość względną, jako że pewne cechy nowego bodźca były zwierzętom znane wcześniej (tunele), ale zmieniła się ich specyfikacja przestrzenna (dodanie tuneli) oraz właściwości (dodanie tunelu ruchomego).

Wyniki tego badania potwierdzają wyniki naszych poprzednich badań (Pisula i in., 2019; Pisula i in., 2021), pokazujące, że nowość ma wartość nagradzającą, która skutkuje

większą aktywnością eksploracyjną szczurów w odniesieniu do nowych obiektów. Należy jednak zwrócić uwagę na dwie kwestie. Pierwsza z nich dotyczy tego, że być może elementy ruchome mogą w pewnym stopniu przyczyniać się do podwyższonego poziomu stresu, ponieważ ich właściwości mogą wywoływać stan niepewności co do konsekwencji zdarzeń związanych z ich eksplorowaniem (Inglis, 2000). Wyniki zdają się wspierać pogląd, że wprowadzenie elementów ruchomych nie ma samo w sobie wystarczającej wartości nagradzającej. Dopiero połączenie ich ze zwiększeniem złożoności przestrzennej przekłada się na wzrost aktywności zachowań eksploracyjnych, co wydaje się wynikiem spójnym z prezentowanym wcześniej badaniem i dostarcza argumentów na rzecz poglądu o dominującej roli informacji przestrzennej w regulacji zachowań eksploracyjnych szczurów.

## 2.3. Czy badanie testem Hole-Board może być pomocne w prognozowaniu zachowań eksploracyjnych szczurów w teście swobodnej eksploracji.

Pisula, W., Modlinska, K., Goncikowska, K., & **Chrzanowska, A**. (2021). Can the Hole–Board Test Predict a Rat's Exploratory Behavior in a Free-Exploration Test? *Animals, 11*(4), 1068.

Celem badania było sprawdzenie, czy przeprowadzenie testu w aparaturze Hole-Board może być pomocne w określeniu stopnia aktywności eksploracyjnej w teście swobodnej eksploracji oraz poddanie analizie trafności pomiaru zachowań eksploracyjnych u szczurów z użyciem testu Hole-Board. Trafność tej metody w zakresie badania zachowań eksploracyjnych została oszacowana za pomocą testu przeprowadzonego w aparaturze służącej do badania swobodnej eksploracji (Pisula i Modlińska, 2020).

Badanie było podzielone na dwie fazy. Pierwsza faza odbywała się w aparaturze Hole-Board, a druga faza przeprowadzona została w omawianej wcześniej aparaturze eksperymentalnej do pomiaru zachowań eksploracyjnych (Pisula i Modlińska, 2020). Hole-Board jest zwyczajowo stosowany do oceny różnych aspektów zdolności poznawczych i emocjonalnych u małych gryzoni, a wywodzi się on z testu otwartego pola (Open Field Test), który najczęściej wykorzystywany jest do badania zachowań lękowych i zachowań eksploracyjnych (Hall i Ballachey, 1934). Aparatura Hole-Board posiada w dolnej części małe cylindryczne otwory, które umożliwiają badanym zwierzętom zanurzenie w nich głowy

i ta aktywność jest miarą zachowania w tej arenie eksperymentalnej. Warto zaznaczyć, że badanie testem Hole-Board jest przedmiotem pewnych kontrowersji związanych na przykład z tym, jak uważają niektórzy autorzy (Hughes, 2007), że wkładanie głowy do otworów może być związane z próbą znalezienia drogi ucieczki, a nie odzwierciedlać zainteresowanie tym co znajduje się w otworach. Jednak test Hole-Board pozostaje jedną z standardowych procedur w badaniach nad różnymi aspektami regulacji zachowania.

W pierwszej fazie badania wzięło udział 80 szczurów: 40 samic i 40 samców. Celem było wyłonienie z tej grupy osobników prezentujących najwyższy (10 samic i 10 samców) i najniższy (10 samic i 10 samców) poziom aktywności eksploracyjnej. Wybrane osobniki przeszły do drugiej fazy eksperymentu czyli do badania zachowań eksploracyjnych w teście swobodnej eksploracji według wspomnianego już niejednokrotnie protokołu (Pisula i Modlińska, 2020). Po fazie habituacji jako nowość wprowadzono zmianę aranżacji przestrzennej (zwiększenie liczby tuneli).

Założenie przyjęte w tym badaniu opierało się na hipotezie, że mechanizmy motywacyjne związane z reakcją na nowość w znajomym środowisku (druga faza badania) jak i aktywność eksploracyjna w teście Hole-Board (pierwsza faza badania) będą podobne. Okazało się jednak, że wyniki uzyskane w teście Hole-Board pozwalały przewidzieć poziom aktywności szczurów w teście swobodnej eksploracji w ograniczonym zakresie. Głównym czynnikiem wyjaśniającym reakcje eksploracyjne w teście swobodnej eksploracji były zmiany, które zachodziły w trakcie trwania eksperymentu, natomiast płeć czy wysokie lub niskie wyniki w teście Hole-Board miały mniejszą wartość predykcyjną.

Warto zwrócić uwagę na kilka aspektów związanych z użyciem tych dwóch aparatów badawczych. Po pierwsze, badanie z użyciem aparatury Hole-Board nie było poprzedzone fazą habituacji (przyjęta procedura była wzorowana na dominującym sposobie wykorzystania Hole-Board obecnym w literaturze przedmiotu). W związku z tym, posługując się terminami zaproponowanymi przez Berlyne'a (1960), można przyjąć założenie, że nowość sytuacji, w której znalazły się zwierzęta podczas badania miała charakter nowości absolutnej. Z kolei rodzaj nowości, który pojawił się w drugiej fazie badania można określić jako nowość względną, gdyż zmieniła się aranżacja przestrzenna znanych wcześniej obiektów. Reakcja na nowość ma tutaj związek z uprzednimi doświadczeniami zwierzęcia (Barto i in., 2013). Kolejnym aspektem jest trafność ekologiczna obu testów. Procedura Hole-Board przeprowadzona została z użyciem światła (75-100 lx), natomiast drugie badanie odbywało się w ciemności. Szczury są zwierzętami nocnymi i zazwyczaj unikają jasno oświetlonych miejsc, w związku z czym badanie ich aktywności w ciemności wydaje się być bardziej

uzasadnione ekologicznie. Badanie w aparaturze Hole-Board było przeprowadzone w warunkach oświetleniowych zbliżonych do tych, jakie panują w pomieszczeniu mieszkalnym szczurów. W zwierzętarni cykl dobowy jest podzielony na 12 godzin z oświetleniem i 12 godzin ciemności. Aktywność zwierząt przypada na czas kiedy światło jest wyłączone. Prowadzenie badania w zbliżonych warunkach oświetleniowych może mieć wpływ na aktywność zwierząt w przestrzeni badawczej. Kolejną istotną kwestią może być przestrzeń aparatury badawczej. Wymiary aparatury Hole-Board są mniejsze niż drugiej aparatury użytej w badaniu, a przestrzeń badawcza to jedna arena, podczas gdy druga aparatura ma trzy oddzielne strefy plus dostęp do transportera. Co prawda w badaniu Hole-Board umożliwiono zwierzętom dostęp do transportera podczas badania, należy się jednak zastanowić czy pomimo tego eksploracja w tej aparaturze nie miała charakteru eksploracji wymuszonej, w przeciwieństwie do aparatury z drugiej fazy badania, która daje możliwość swobodnej eksploracji. Analiza wyników z dwóch przeprowadzonych badań wskazuje na to, że badanie w aparaturze Hole-Board w małym stopniu pozwoliło przewidzieć poziom aktywności zwierząt w drugim badaniu. Wygląda na to, że inne mechanizmy regulacji zachowania, takie jak reaktywność emocjonalna czy ocena ryzyka, mogą odgrywać większą rolę w kształtowaniu się aktywności zwierzęcia w aparaturze Hole-Board.

## 2.4. Wpływ bodźców świetlnych na zachowania eksploracyjne szczurów w teście swobodnej eksploracji

Pisula, W., Modlinska, K., Goncikowska, K., & **Chrzanowska, A**. (2022). Decrease in the rewarding value of spatial novelty due to the contamination of the stimulus field with light–Evidence from a free exploration test involving rats. *Behavioural Processes*, *202*, 104738.

Badania na szczurach opisane w niniejszej pracy, prowadzone według wspomnianego wcześniej protokołu (Pisula i Modlińska, 2020), odbywały się w całkowitej ciemności. Celem tego było narażanie szczurów na jak najmniejszy stres podczas procedury badawczej. Dodatkowo dało to możliwość wprowadzenia bodźców świetlnych jako źródła nowości. Celem tego badania było sprawdzenie reakcji zwierząt na zmianę aranżacji przestrzennej wraz z wprowadzeniem bodźców świetlnych. Już w latach 40 XX w. wykazano, że ekspozycja na światło może wywoływać reakcję awersyjną u szczurów (Keller, 1941). Potwierdzają to również badania nad reakcjami lękowymi (Bouwknecht i in., 2007; Garcia i

in., 2005, Kuniishi, 2017). Z drugiej strony jednak są badania, które wykazały, że stymulacja białym światłem może mieć wartość nagradzającą (Kish, 1955). Zjawisko to zostało nazwane wzmocnieniem sensorycznym (Barnes i in, 1959; Kish, 1955).

W badaniu wykorzystano trzy grupy szczurów. W każdej grupie w fazie testowej dodano tunele w takim samym ustawieniu. Poza tym w dwóch grupach dodano bodźce świetlne (przyćmione białe światło) w tunelach w środkowej części. W jednej grupie natężenie światła wynosiło 25-30 lx (oznaczone jako "LowLight"), w drugiej zaś 55-65 lx (oznaczone jako "HighLight"). W trzeciej grupie nie użyto bodźców świetlnych ("NoLight")

Wyniki badania pokazały, że szczury ze wszystkich trzech grup spędzały więcej czasu w strefie, gdzie wprowadzono zmianę. Może to wskazywać na preferencję przebywania w bardziej złożonej i - co za tym idzie - atrakcyjniejszej strefie. Szczury z grup NoLight i LowLight przejawiały wzrost czasu trwania interakcji z tunelami w zmienionej strefie we wszystkich dniach testowych, szczury z grupy HighLight nie wykazywały tego efektu. Szczury z grupy NoLight wykazały spadek częstotliwości interakcji z tunelami w niezmienionej strefie, natomiast u szczurów z pozostałych grup tego nie zaobserwowano. Ten wynik pokazuje preferencję tej komory, gdzie wzrosła złożoność środowiska w grupie NoLight. Natomiast w grupach LowLight i HighLight czas przebywania w niezmienionej (ciemnej) strefie nie zmniejszył się. W odniesieniu do złożoności środowiska może to odzwierciedlać działanie mechanizmów regulacyjnych utrzymujących poziom złożoności docierających bodźców na względnie stałym poziomie. Może to wskazywać na względną atrakcyjność strefy ciemnej, choć niezmienionej. Stefa ze zmianą aranżacji tuneli i światłem z jednej strony była atrakcyjna, ponieważ nastąpił wzrost złożoności środowiska, jednak bodźce świetlne mogły powodować potrzebę regulacji intensywności docierających bodźców, dlatego też nie zaobserwowano spadku czasu przebywania zwierząt w komorze ciemnej.

Badanie pokazało różnice między grupami z różnych warunków oświetleniowych. Szczury z grupy LowLight spędzały więcej czasu na kontakcie z tunelami, natomiast szczury z grupy HighLight mniej. Może to oznaczać, że intensywniejsze oświetlenie było jednak bardziej awersyjne. Jednak nie na tyle, by szczury unikały przebywania w tej strefie, jedynie powstrzymały się od bezpośredniego kontaktu z oświetlonymi tunelami. Okazuje się więc, że intensywność światła, nawet utrzymywana w niskim zakresie, jest istotnym aspektem stymulacji. Należy pamiętać, że w zależności od okoliczności, stymulacja światłem może odgrywać rolę apetytywną lub awersyjną. W badaniu zostało zastosowane światło o niskiej intensywności, dlatego można założyć, że właściwości nieskostresowe areny doświadczalnej

zostały zachowane. W większości badań z użyciem światła jego natężenie było znacznie wyższe (por. Godsil i Fanselow, 2004). Stymulacja białym światłem może mieć wartość nagradzającą, co mogą sugerować wyniki uzyskane w grupie LowLight.

## 2.5. Badanie reakcji na nowość u żółwi lądowych.

**Chrzanowska, A**., Modlinska, K., Stryjek, R., & Pisula, W. (2015). Response to Perceptual Novelty in Tortoises-A Preliminary Study. *Journal of Biology and Life Science*, *7*(1), 12-18.

Ostatnim z cyklu prezentowanych badań jest wstępne badanie reakcji na nowość przeprowadzone na dwóch gatunkach żółwi lądowych. Badania zachowań eksploracyjnych i reakcji na nowość najczęściej prowadzi się na gatunkach należących do ssaków i ptakach. Zważywszy jednak na pewne podobieństwa dotyczące mechanizmu działania mózgu u owodniowców (kręgowców, u których rozwój zarodkowy występuje na lądzie) (Broglio, i in., 2015; Salas i in., 2003) warto prowadzić badania nad tymi zachowaniami również na gadach. Prowadzenie badań porównawczych na różnych gatunkach kręgowców należących do owodniowców pozwoli prześledzić ewolucję wyższych czynności psychicznych.

Zainteresowanie badaczy zachowaniem gadów nie jest niczym nowym. W 1904 Yerkes badał percepcję przestrzenną u żółwi, a w latach 30 XX w. Tinklepaugh prowadził badania nad uczeniem się na żółwiach (Tinklepaugh, 1932).

W prezentowanym badaniu wykorzystano trzy żółwie: dwa z gatunku *Testudo hermanni* oraz jednego z gatunku *Agrionemys horsfieldi*. Aparatura badawcza była zaprojektowana tak, by umożliwić zwierzętom oglądanie obiektów z niedużej odległości, jednak nie mogły one ich dotykać. W każdej sesji każdy żółw miał możliwość obserwowania trzech obiektów. Dwa z nich były stałe: jeden obiekt, imitujący bodziec znaczący biologiczne (imitacja sałaty) oraz obiekt neutralny, nie aktywizujący napędu pokarmowego (żółta kula). Trzeci obiekt w każdej sesji był zmieniany (elementy w różnych kolorach i kształtach). Miarą reakcji na nowość był czas i częstotliwość wpatrywania się w każdy obiekt.

Należy zaznaczyć, że badanie to miało charakter wstępny i eksploracyjny. Wyniki wskazują na istnienie stałego wzorca u wszystkich trzech badanych żółwi. Obiekt neutralny wzbudzał najmniejsze zainteresowanie zwierząt. Dwa pozostałe obiekty wzbudzały reakcję wpatrywania się. Obiekt imitujący zieloną roślinę jako obiekt o znaczeniu biologicznym

wzbudzał długo trwające i częściej występujące reakcje. Natomiast zmienny bodziec za każdym razem wzbudzał silniejsze reakcje niż bodziec neutralny. U badanych żółwi wystąpiła reakcja, którą można interpretować w dwojaki sposób. Po pierwsze, można założyć, że obiekt imitujący pokarm był dobrze zaprojektowany i istotnie przykuwał uwagę zwierząt jako potencjalny pokarm. Obiekt sam w sobie był nowy, ale jego właściwości (zielony kolor, kształt) były prawdopodobnie cechami bodźca kluczowego wzbudzającego bezwarunkową/instynktowną reakcję popędową, zgodnie z postulatami klasycznej etologii zwierząt (Lorenz, 1981). Obiekty zmiennie wpływały na zachowanie się zwierząt przez swoją nowość, ponieważ żółwie nigdy wcześniej nie miały styczności z bodźcami o takich właściwościach, a były dobierane tak, aby uniknąć uzyskania wpływu podobnego do tego wywieranego przez imitację sałaty. Badanie wykazało, że żółwie są wrażliwe na nowość stymulacji oraz, że nowość stymulacji motywuje je do zachowań w kierunku źródła postrzeganej nowości.

# 3. Ograniczenia

Badania przedstawione w tym cyklu ukazały interesujące wyniki dotyczące zachowań eksploracyjnych i reakcji na nowości, nie są one jednak wolne od ograniczeń. Trzeba zwrócić uwagę na fakt, że badania zostały przeprowadzone na przedstawicielach dwóch gromad: gadów i ssaków (i tylko trzech gatunkach: szczurach i dwóch gatunkach żółwi). W przyszłości na pewno warto byłoby przeprowadzić badania na innych gatunkach gadów, ptaków i ssaków, aby uzyskać pełniejszy obraz tego jak ewolucyjnie kształtowały się zachowania eksploracyjne i reakcja na nowość wśród owodniowców.

Analizując wyniki badania, w którym hodowano szczury w warunkach wzbogacenia stałego i wzbogacenia zmiennego warto zastanowić się nad kluczowymi właściwościami wzbogacenia środowiska - czy było to wprowadzenie obiektów do areny mieszkalnej i zmienność ich aranżacji lub jej brak, czy może sam fakt, że rozmiar klatki hodowlanej był znacznie większy niż zwyczajowa klatka laboratoryjna (Manosevitz i Pryror, 1975).

W badaniu z udziałem żółwi lądowych istotnym ograniczeniem jest liczba badanych zwierząt. Dlatego też można było wskazać jedynie wstępny charakter tego badania zachęcający do dalszych poszukiwań.

W badaniu z obiektami ruchomymi obiektami wątpliwości może budzić wybór wprowadzenia elementu ruchu poprzez użycie ruchomych tuneli. Jak wspomniano wcześniej

szczury musiały wykryć ruch tunelu poprzez wejście na niego co powodowało wykrycie tej zmiany z pewnym opóźnieniem (brak motywacji do eksploracji po zastaniu pozornie niezmienionych właściwości tuneli).

# 4. Podsumowanie i dalsze kierunki badań

Wyniki przedstawionych badań nad wpływem złożoności środowiska na zachowanie pokazały pewne wspólne właściwości zachowania oraz pewne różnice zależne od rodzaju zastosowanej manipulacji. We wszystkich badaniach z udziałem szczurów wzrost złożoności środowiska przełożył się na zwiększenie aktywności eksploracyjnej. Warto zwrócić uwagę na fakt, że badania te były zaprojektowane tak, aby umożliwić zwierzętom swobodną eksplorację. We wszystkich badaniach na szczurach pojawił się również rodzaj zachowania eksploracyjnego, który można określić za Berlyne'm (1960) jako *specific exploration* czyli zachowania ukierunkowanego na badanie nowych obiektów. Wyniki potwierdziły również występowanie prawidłowości wymienionych przez Gleznera (1958), na przykład to, że szczury preferują miejsca, gdzie występują bodźce bardziej złożone oraz że możliwość podjęcia eksploracji ma wartość nagradzającą. W badaniu, w którym użyto ruchomych tuneli oraz w badaniu gdzie użyto bodźców świetlnych wyniki dotyczące reakcji na nowość i aktywności eksploracyjnej były bardziej zróżnicowane. Ruchome tunele same w sobie nie dostarczyły wystarczającej stymulacji, dopiero połączenie zwiększenia liczby elementów z dodaniem tunelu ruchomego ujawniało zmiany w zachowaniu. Należy jednak pamiętać, że elementy ruchome mogły być też czynnikiem stresującym. Podobnie rzecz ma się w badaniu z użyciem bodźców świetlnych, gdzie nowy bodziec miał jednocześnie właściwości przyciągające (przebywanie w strefie gdzie wprowadzono zmianę) oraz lekko awersyjne (unikanie bezpośredniego kontaktu z oświetlonymi tunelami w grupie w wyższym natężeniem światła).

We wszystkich badaniach, łącznie z badaniem na żółwiach lądowych, wyniki pokazały preferencję nowych bodźców pojawiających się w otoczeniu.

Jeśli chodzi o dalsze kierunki badań, ciekawym wydaje się dalsza eksploracja tematu obiektów ruchomych i ich oddziaływania na zachowanie szczurów. Być może trzeba by się zastanowić nad innego typu elementami, które dawałby zwierzętom możliwość manipulowania nimi, ale na przykład nie wymagałby tego, żeby trzeba było na nie wejść i

wprawić je w ruch. Być może w kolejnym badaniu należy w aparaturze badawczej umieścić mniejsze elementy, które będą dawały szczurom większe poczucie kontroli nad sytuacją i większą możliwość regulacji stymulacji płynącej z obiektu.

Dalsze prace mogłyby zostać zaprojektowane w oparciu o wystandaryzowane procedury badawcze, pozwalające na szczegółową analizę porównawczą zachowania się zwierząt konfrontowanych ze zmianami środowiskowymi. Taki program badawczy powinien umożliwić weryfikowanie hipotez szczegółowych dotyczących procesów poznawczych zwierząt związanych z przetwarzaniem zmienności i złożoności środowiska.

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# The impact of changeability of enriched environment on exploration in rats

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ABSTRACT

Although the positive effect of environmental enrichment on animals' cognitive capacities is well-known, it remains unclear what role changeability plays in this context. Our study aims to analyse the impact of environmental changeability on the level of exploration and the rate of habituation to novelty.

Prior to the experiment, the animals were housed in three settings: enriched stable conditions, enriched changing conditions and standard conditions. Environmental changeability was introduced by re-arranging objects in the housing pen. A test was conducted to measure the level of exploration in adult individuals.

The study results suggest that rats housed in standard conditions exhibit a higher demand for interactions with the new environment. However, once novelty is introduced, rats from the enriched environments spend more time than their standard counterparts exploring the new objects. No significant differences have been observed in the behaviour of rats from the stable and changeable conditions. It may be concluded, therefore, that in a setting characterised by long-lasting environmental enrichment, the changeability of the environment plays no major role, at least with respect to exploration, general activity and the rate of habituation to novelty. It may be linked to the relatively quick extinguishment of behaviours reinforced by intrinsic reinforcement.

## 1. Introduction

In laboratory settings, environment can be enriched by providing the animal with a cognitively, physically and socially stimulating living space which enables spontaneous exploration (Baroncelli et al., 2010). Enrichment is often achieved by the introduction of objects (that is, wooden blocks, swings, houses, spinning wheels), as well as more opportunities for social contact (e.g., Bloomsmith et al., 1991; Newberry, 1995), which make the basic settings more complex and more diversified. From an ecological perspective, environmental enrichment results in a larger scope of affordances of the environment available to the animal (for affordances see Gibson, 2014; Rietveld and Kiverstein, 2014). Numerous studies show that animals maintained in enriched conditions perform better in learning tasks (also see e.g.: Gardner et al., 1975; Schrijver et al., 2002), and demonstrate a higher level of exploratory activity and lower anxiety (e.g., Gardner et al., 1975; Genaro and Schmidek, 2001; Pietropaolo et al., 2004). Moreover, environmental complexity increases novelty-seeking behaviour (Fernandez-Teruel et al., 1997) and object exploration (Widman and Rosellini, 1990), while reducing anxiety-like behaviour and increasing activity. What is more, increased environmental complexity evokes changes in behaviour that have been linked to various changes in the brain (e.g., Hebb, 1946; Benaroya-Milshtein et al., 2004; Kolb and Whishaw, 1998; Lewis, 2004; Rosenzweig and Bennett, 1996). Environmental enrichment has a significant impact on the nervous system of both young and mature animals (e.g., Frick and Fernandez, 2003; Camel et al., 1986), may contribute to the reversal of cognitive and emotional impairments (e.g., Dahlqvist et al., 2004; Francis et al., 2002; Jankowsky et al., 2005), and can have a positive effect on animal welfare in captivity (e.g., Abou-Ismail et al., 2010).

Standard environmental enrichment in laboratory conditions often remains constant throughout the entire period of environmental manipulation (e.g. exposure to the enrichment). There are relatively few studies investigating the impact of environmental changeability occurring in those kind of setups. In the natural environment, animals encounter a wide range of environmental stimuli that may change in a variety of ways (eg. Wasserman et al., 2004). This changeability may have a significant impact on their behaviour and may influence different groups of animals to varying degrees. For instance, domesticated animals bred in a stable laboratory or farm environment may react to environmental changeability in a different way than their wild counterparts. The changeability of the living environment may also affect the development of based on previous experience animal expectations for the surroundings to be less or more predictive and controllable. Since the motivation to control environmental events seem to be an important element of animal and human behaviour regulation, the

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generalized experience of unpredictability vs stability may have profound effect on animal cognitive development in general (Bassett and Buchanan-Smith, 2007; Chorpita and Barlow, 1998; Cramer et al., 1997; Job, 2006).

Introducing frequent changes to an animal's environment forces the animal to explore and to continually update internal representations of its environment (Leonard and McNaughton, 1990; Poucet and Benhamou, 1997). The changes affect not only the structure of the environment and the location of objects, but also the location of food sources and the type of food. The unpredictability of changes and the lack of control over the surroundings trigger a stress response (LaDage, 2015). One way of dealing with the constant presence of stimuli resulting from such changes and their unpredictability may involve moderation of the emotional reaction to novelty. This hypothesis is confirmed by the data obtained in a study where rats kept in standard conditions were exposed to changes in experimental setup in every trial (Pisula et al., 2006). However, in individuals with a high level of emotional reactivity, constant change may result in the worsening of cognitive functions (Benus et al., 1987). On the other hand, as Staddon (2016, p. 204) states, "states of nature that make no difference, […], will not usually be differentiated — will not produce different states of the animal." It may follow from that constant changes which are associated with neither reward nor punishment result in decreased sensitivity to the novelty encountered in the environment and do not trigger an emotional response. Even if we assume that constant change is an intrinsic reinforcement leading to increased exploration, intrinsically reinforced, unlearned behaviours such as curiosity habituate both after the introduction of novelty and across consecutive introductions of novelty (Tarou and Bashaw, 2007).

Although the positive effect of environmental enrichment on an animal's cognitive capacities is well-known and widely documented, it remains unclear what precise role environmental changeability plays in the context of environmental enrichment. The current state of knowledge fails to suggest a clear direction of the relationship between such stimuli and later behaviour. Our study aims to analyse the impact of environmental changeability on the level of exploration in rats and the rate of habituation to novelty in the environment explored. The changeability of the enriched environment was ensured in our experiment by changing the arrangement of the objects in the pen on a daily basis.

## 2. Materials and methods

### 2.1. Subjects

The sample was comprised of 29 male rats of the Lister Hooded stock (11 rats for enriched stable conditions group; 10 rats for enriched changeable conditions group; and 8 rats for standard condition). The animals were sourced from Charles River, Germany, via AnimaLab Sp. z o.o., Poland. The rats were housed in the vivarium of the Institute of Psychology, Polish Academy of Sciences, Warsaw, Poland.

### 2.2. Housing conditions

Prior to the experiments, the rats were kept in three different housing setups. The first group was reared in an enriched stable environment. The second group – in an enriched, but constantly changing environment. The third group was reared under standard conditions (control group). The rats were randomly assigned to one of the three sets of conditions. All rats were housed in groups consisting of 4–6 individuals. The groups' configuration was unchanged during the study, so as to maintain a stable social environment for all animals (see Tanaś and Pisula, 2011; Pisula et al., 1992).

#### 2.2.1. Enriched stable conditions (ESC)

The housing area was a pen with combined dimensions of approx. 2000 mm × 1000 mm × 1000 mm, which enabled the rats to move freely in three dimensions. The pen was covered with wire mesh placed on top of a wooden frame. The floor was covered with dust-free softwood granules "Tierwohl Super©". The pen was equipped with objects that enriched the housing environment: a wooden box with two entrances, which provided shelter and a nesting site; two wooden tunnels; three pillar-shaped wooden blocks; a spool of hemp twine suspended on a metal chain; a horizontal wooden bar placed on two posts connected to a ramp covered with wire mesh; a mirror; and a wooden wall covered with a metal net. In addition, inside the pen an open standard breeding cage (Tecniplast© Eurostandard Type IV) was placed. It was fitted with three dispensers (two with water, one with fodder) inside the cage. The purpose of placing on open cage inside the pen was to habituate the animals to the cage and its interior to avoid the novelty effect which would have been induced by putting the animals in that cage before the experiment. The scheme of the housing pen is shown in Fig. 1. The animals were fed standard laboratory fodder (Labofeed H, WP Morawski, Kcynia, Poland), which was put in two places (inside the cage and in a metal bowl outside the cage). The area was also fitted with additional water dispensers.

**Fig. 1.** Housing pen with objects used for environmental enrichment.

#### 2.2.2. Enriched changing conditions (ECC)

The housing conditions were the same as described in Enriched stable conditions (ESC). However, in this setup, the objects inside the pen were placed in different configurations and locations every day. Similarly to ESC conditions, the animals were fed standard laboratory fodder (Labofeed H, WP Morawski, Kcynia, Poland), which was put in two places (inside the cage and in a metal bowl outside the cage, but the metal bowl was shifted daily). Additional foods were given to the rats to ensure food changeability including: oat flakes, rice flakes, corn flakes, apples and carrots. These types of food were provided individually or in different combinations; they were also sometimes mixed with herbs (dried parsley, coriander, basil, dill). Additionally, the location and type of food was changed daily. We decided not to introduce new objects, which can increase the variability of the environment during the rearing phase, because new objects could have different properties

(e.g., higher potential for motor activities) than objects from the stable environment. It would add another variable in the study and prevented the comparison of the impact of both environments on rats.

*2.2.3. Standard conditions (SC)*

The rats were housed in groups of 4 in Tecniplast© Eurostandard Type IV cages (610 mm × 435 mm × 215 mm) with dust-free softwood granules Tierwohl Super© as bedding and with ad libitum access to water and standard laboratory fodder (Labofeed H, WP Morawski, Kcynia, Poland).

In each of the housing setups, the day/night cycle was set at 12/12 h, with the lights-on at 8 A M; the temperature was maintained at a constant 21–23 °C. The pen arena was lit with fluorescent lamps at 75–100 lx (depending on the location). The cages and pens were cleaned once a week, on the same day and at a fixed time. Fodder and water were replenished daily in all housing setups. All rats kept in our laboratory were housed, bred and taken care of in accordance with the Regulation of the Polish Minister of Agriculture and Rural Development of 14 December 2016 on laboratory animal care; the experimental procedures were approved by the 1 st Local Ethics Committee on Animal Experimentation in Warsaw, Poland.

The animals' behaviour inside the pens was monitored by means of a video camera, and the changes of food and arrangement of the objects were recorded in photos and reports.

*2.3. Procedure*

Prior to the experiment, at 23 PND, the animals were put in the housing pen. They were kept in one of the experimental setups for a period of three months. The rats kept in standard conditions were put in cages after the end of the weaning stage (at 23 PND), and remained there until the onset of the experiment.

The aim of the exploration test was to compare the process of investigating a new environment, the rate of habituation to it, and the reaction to the introduction of a novelty of low intensity into a well-known context. The apparatus and measurement methods were similar to those used in our previous studies (Pisula, 2003, 2004; Pisula and Siegel, 2005; Pisula et al., 2006; Tanaś and Pisula, 2011; Pisula et al., 2012). The reason for using this apparatus in the present study was that, contrary to most other tests (eg. Open Field Test, Hole Board etc.), it enables a lateral view. This way of observing the animal allows for a detailed observation of its behaviour, which is crucial for detecting emotional reactivity in animals (e.g. grooming).

The experimental chamber (Fig. 2) was a box measuring 800 mm × 600 mm × 800 mm. The chamber was divided into three zones: A, B, and C by two walls running perpendicularly to its longer side. The front of the chamber was a transparent wall which could be lifted to obtain full access to the experimental arena. The wooden division walls between the zones had triangular entrances (120 mm × 140 mm) at the bottom, which enabled free movement between the chamber parts. The entire chamber was covered with a layer of washable varnish. There were two tunnels (200 mm × 120 mm × 80 mm) in zones B and C made of hardwood covered with washable paint. In contrast to the most frequently used two-dimensional experimental settings (eg. Open Field Test, Hole Board etc.), where animals explore flat surfaces, these tunnels provide a complex three-dimensional environment. The animal has the possibility not only of exploring the surface of the experimental arena, but also of climbing and going into the tunnels. The central zone (A) was left empty – there was a hole curved in the back wall of the chamber which served as an entrance for animals going from the transporter into the chamber.

At the start of each trial, a small cylindrical cage (the 'starting box' – 60 mm in diameter with doors 120 mm high and 100 mm wide) with the tested animal inside was placed by the entrance to zone A. The entrance door was then opened and it was left open until the end of the test. The animal was free to stay in the starting box or leave it to explore

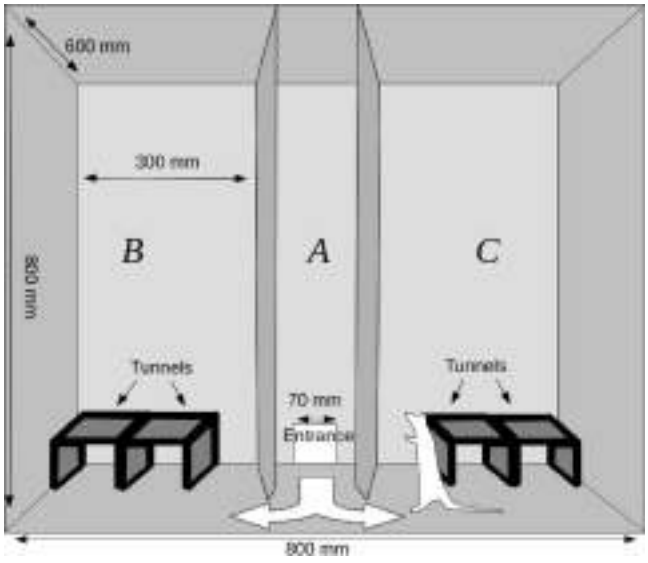


**Fig. 2.** Experimental chamber used for investigating exploratory behaviours. A - the central zone of the experimental chamber with an entrance to the apparatus; B - the left zone of the experimental chamber (no changes throughout the experiment); C - the right zone of the experimental chamber (novelty in the form of additional tunnels was introduced in this zone - see Fig. 3). The curved arrows at the bottom of the figure show the direction in which rats can move between the zones through the passages cut in the inside walls.

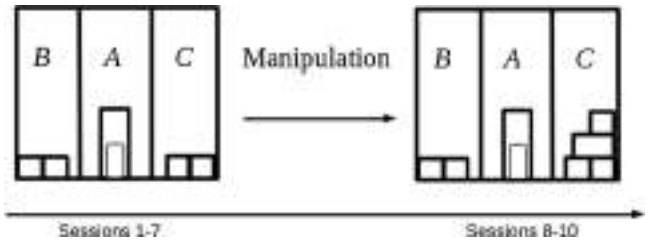


**Fig. 3.** Arrangement of objects in the experimental chamber during the experiment.

the chamber. The first seven trials were habituation trials during which the apparatus was arranged in the same way (Fig. 3). The introduction of novelty (i.e. the addition of new two tunnels on top of the old ones in zone C) took place between trials 7 and 8. The three subsequent trials were conducted with the chamber in this new arrangement (Fig. 3). Each trial was 7 min long and was conducted for each animal once a day.

A video camera was placed approximately 1.5 m away from the transparent front wall of the experimental chamber. The camera was set in the night-shot mode to enable filming in the dark. Behaviours observed were coded on the basis of the recorded material using the BORIS event logging software (Friard and Gamba, 2016). This program makes it possible to define particular behaviours and to score the time and frequency of selected behaviours. In this study, we scored selected behaviours occurring during the entire experimental session. As a result, the exact time of individual bouts of behaviours, their frequency and, consequently, the total time spent engaging in a given behaviour were assigned specific scores. The behaviours analysed comprised the following: latency to leave the starting box; amount of time spent in the starting box; total time spent in the unchanged zone of the chamber; total time spent in the changed zone of the chamber; time spent on contact with the tunnels in the unchanged zone of the chamber; and total time spent on contact with the tunnels in the changed zone of the chamber. As a measure of stress response, the amount of time each rat spent on grooming was assessed (D'Aquila et al., 2000; van Erp et al.,

1994; Katz et al., 1981; Komorowska and Pisula, 2003; Thor et al., 1988).

### 2.4. Data presentation and statistical analyses

To enhance the legibility of the results, graphs, and tables, two phases were marked out from among all habituation trials: phase H1/H2, which involved the initial measurement of behaviour, that is, the animals' behaviour at the start of the experiment; and phase H6/H7, which measured the effect of habituation to the experimental conditions. Subsequently, all test trials were divided into two distinct phases: T1, when novelty was introduced (i.e. the additional tunnels in zone C); and phase T2/T3, which reflected the level of rats' exploratory behaviour during trials conducted after the introduction of novelty (measuring the level of habituation to change).

The data was analysed using a General Linear Model procedure (GLM), with the housing setups (ESC, ECC and SC) as the between-subject factors, and repeated measurements (H1/H2, H6/H7, T1, T2/T3) as the within-subject factor. Differences were considered significant for p values of $\leq 0.05$.

## 3. Results

### 3.1. Time spent in zone A (central)

The amount of time spent in zone A (the central zone of the experimental chamber) was measured.

The analysis showed a significant phase by housing setups interaction (Wilks' Lambda; $F(1,28) = 3.677$; $p = 0.004$; $eta^2 = 0.306$), and a significant main factor effect for the phase (Wilks' Lambda - $F(1,27) = 81.985$, $p \leq 0.001$; $eta^2 = 0.908$).

Analysis of variance (ANOVA) was used to analyse the differences in time spent in zone A by individuals from the three housing setups during each phase; it yielded differences between the groups only in phase H1/H2 ($F(2,29) = 10.160$, $p = 0.001$; $eta^2 = 0.429$) - Fig. 4. Post hoc analysis using the Tukey HSD test showed that in the habituation phase H1/H2, the ECC rats spent more time in the central zone than the SC rats ($p \leq 0.001$; $M_{ECC} = 146.2$, $SD_{ECC} = 23.2$; $M_{SC} = 106.6$, $SD_{SC} = 18.7$; Cohen's $d = 1.891$).

A paired samples Student's *t*-test was used to assess the differences in time spent in zone A by animals from the three housing setups between individual phases. In ECC rats, there was a decrease in the amount of time spent in this zone in phase H6/H7 ($t(9) = 2.791$, $p = 0.021$; Cohen's $d = 0.884$), followed by another decrease in the first trial after the introduction of novelty, that is, in phase T1 ($t(9) = 14.325$, $p \leq 0.001$; Cohen's $d = 4.534$), which was subsequently followed by an increase in phase T2/T3 ($t(9) = -2.740$, $p = 0.023$; Cohen's $d = 0.873$). In the ESC group, however, the statistically significant aspect was the decrease in the amount of time spent in the central zone in phase T1 ($t(9) = 6.839$, $p \leq 0.001$; Cohen's $d = 2.160$), followed by an increase in phase T2/T3 ($t(9) = -3.021$, $p = 0.014$, Cohen's $d = 0.966$). In the SC group, a decrease was observed in phase T1 only ($t(9) = 3.826$, $p = 0.004$; Cohen's $d = 1.210$).

### 3.2. Time spent in zone B (left)

The amount of time spent in zone B (the left zone of the experimental chamber) was measured.

The analysis showed a significant phase by housing setups interaction (Wilks' Lambda; $F(2,27) = 2.848$; $p = 0.018$; $eta^2 = 0.255$), and significant phase differences (Wilks' Lambda; $F(1,27) = 26.516$; $p \leq 0.001$; $eta^2 = 0.761$).

Analysis of variance (ANOVA) was used to compare the amount of time spent in zone B by individuals from the three housing setups during each phase; it yielded differences between the groups only in phase H1/H2 ($F(2,29) = 16.423$, $p \leq 0.001$; $eta^2 = 0.549$) – Fig. 5. Post hoc analysis using the Tukey HSD test showed that in phase H1/H2, standard rats spent more time in the left zone than ESC rats ($p \leq 0.001$; $M_{SC} = 155.5$, $SD_{SC} = 28.2$; $M_{ESC} = 94.3$, $SD_{ESC} = 22.5$; Cohen's $d = 2.242$) and ECC rats ($p = 0.001$; $M_{ECC} = 111.3$, $SD_{ECC} = 22.7$; Cohen's $d = 1.747$). No significant differences between the groups were observed in the other phases.

A paired samples Student's *t*-test was used to assess the changes in the amount of time spent in zone B by animals from different housing setups between individual phases. In ECC rats, there was a marked decrease in phase T1 ($t(9) = 2.853$, $p = 0.019$, Cohen's $d = 0.902$), that is, after the introduction of novelty to zone C. In ESC rats, there was a significant increase in phase H6/H7 ($t(9) = -2.602$, $p = 0.029$, Cohen's $d = 0.823$), followed by a decrease in phase T1 ($t(9) = 10.738$, $p \leq 0.001$, Cohen's $d = 3.396$), which was then followed by another increase in T2/T3 ($t(9) = -3.083$, $p = 0.013$, Cohen's $d = 0.975$). In the SC group, there was a marked decrease in the time spent in the left zone in phase H6/H7 ($t(9) = 3.842$, $p = 0.004$, Cohen's $d = 1.215$), as well as in phase T1 ($t(9) = 3.531$, $p = 0.006$, Cohen's $d = 1.117$).

### 3.3. Time spent in zone C (right)

The amount of time spent in zone C (the right zone of the

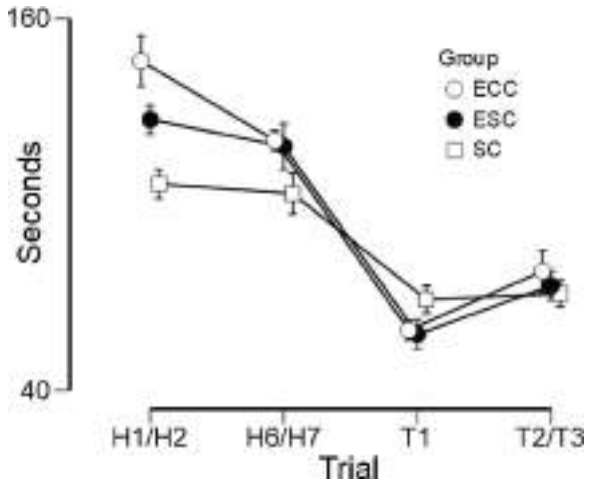


**Fig. 4.** Time (s) spent by rats in the central zone of the experimental chamber. ECC - Enriched changing conditions; ESC - Enriched stable conditions; SC - Standard conditions.

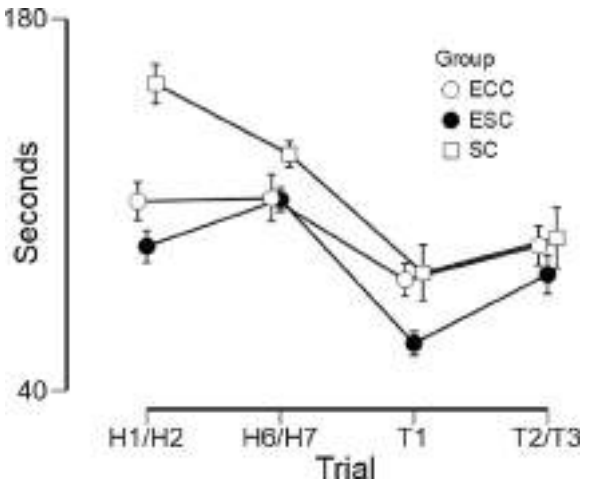


**Fig. 5.** Time (s) spent by rats in the left zone of the experimental chamber. ECC - Enriched changing conditions; ESC - Enriched stable conditions; SC - Standard conditions.

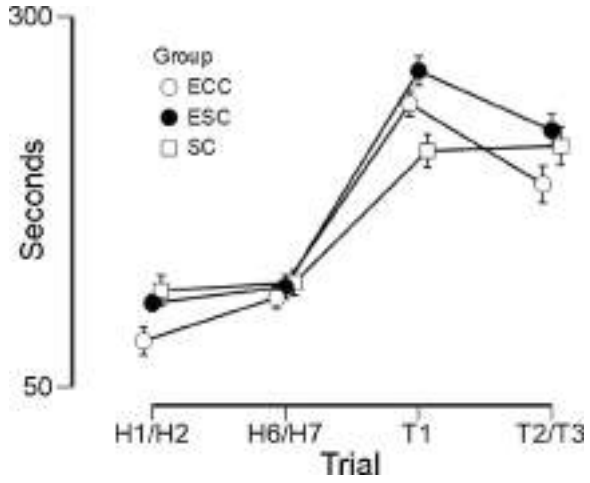


**Fig. 6.** Time (s) spent by rats in the right zone of the experimental chamber. ECC - Enriched changing conditions; ESC - Enriched stable conditions; SC - Standard conditions.

experimental chamber) was measured.

The analysis showed a significant phase by housing setups interaction (Wilks' Lambda; $F(1,28) = 2.774$, $p = 0.021$; $eta^2 = 0.250$), and a significant main factor effect for the phase (Wilks' Lambda - $F(1,27) = 100.134$, $p \leq 0.001$; $eta^2 = 0.923$).

Analysis of variance (ANOVA) was used to analyse the time spent in zone C by individuals from the three housing setups during each phase; it yielded differences between the groups in phase H1/H2 ($F(2,29) = 6.079$, $p = 0.007$; $eta^2 = 0.310$), and in phase T1 ($F(2,29) = 6.589$, $p = 0.005$; $eta^2 = 0.328$) – Fig. 6. Post hoc analysis using the Tukey HSD test showed that in the habituation phase H1/H2, ECC rats spent less time in the right zone than their ESC counterparts ($p = 0.045$; $M_{ECC} = 80.9$, $SD_{ECC} = 20.0$; $M_{ESC} = 106.6$, $SD_{ESC} = 24.5$; Cohen's d = 1.148) and SC rats ($p = 0.007$; $M_{SC} = 114.9$, $SD_{SC} = 23.4$; Cohen's d = 1.623). In phase T1, ESC rats spent more time in zone C than SC rats ($p = 0.003$; $M_{ESC} = 263.4$, $SD_{ESC} = 33.1$; $M_{SC} = 209.4$, $SD_{SC} = 36.1$; Cohen's d = 1.563).

A paired samples Student's *t*-test was used to assess the changes in the amount of time spent in zone C by individuals from different housing setups between individual phases. In ECC rats, there was an increase in the time spent in the right zone in phase H6/H7 ($t(9) = -3.103$, $p = 0.013$; Cohen's d = 0.981), followed by a marked increase in the first phase after the introduction of novelty, that is, in T1 ($t(9) = -11.934$, $p \leq 0.001$; Cohen's d = 3.774), after which there was a decrease in phase T2/T3 ($t(9) = 3.810$, $p = 0.004$; Cohen's d = 1.205). In ESC rats, however, the statistically significant aspect was the increase in the amount of time spent in zone C in T1 ($t(9) = -12.338$, $p \leq 0.001$; Cohen's d = 3.902), followed by a decrease in phase T2/T3 ($t(9) = 2.486$, $p = 0.035$; Cohen's d = 0.786). In SC rats, there was an increase in phase T1 ($t(9) = -5.580$, $p \leq 0.001$; Cohen's d = 1.764).

### 3.4. Time spent in transporter

The amount of time spent in the transporter, excluding the latency to leave the transporter (that is, the amount of time from the moment the transporter was opened until the rat first entered the experimental apparatus) was measured.

The analysis showed a significant phase by housing setups interaction (Wilks' Lambda; $F(2,27) = 4.225$; $p = 0.002$; $eta^2 = 0.336$), and significant phase differences (Wilks' Lambda; $F(1,27) = 16.735$; $p \leq 0.001$; $eta^2 = 0.668$).

Analysis of variance (ANOVA) was used to analyse time spent in transporter by individuals from the three housing setups during each phase; it yielded differences between the groups only in phase H1/H2 ($F(2,29) = 12.318$, $p \leq 0.001$; $eta^2 = 0.477$). Post hoc analysis using the Tukey HSD test showed that in the first phase, SC rats spent less time in the transporter than ESC rats ($p \leq 0.001$; $M_{SC} = 42.9$, $SD_{SC} = 16.9$; $M_{ESC} = 87.6$, $SD_{ESC} = 26.2$; Cohen's d = 2.048) and ECC rats ($p = 0.001$; $M_{ECC} = 84.9$, $SD_{ECC} = 23.5$; Cohen's d = 2.019). No differences between the groups were observed in the other phases.

A paired samples Student's *t*-test was used to assess the changes in the amount of time spent in the transporter by individuals from different housing setups between individual phases. In ECC rats, there was a significant decrease in the amount of time spent in the transporter in phase H6/H7 ($t(9) = 2.609$, $p = 0.028$; Cohen's d = 0.825). In SC rats, however, an increase was observed in phase H6/H7 ($t(9) = -3.628$, $p = 0.006$; Cohen's d = 1.147). In the ESC group, there was a marked decrease in T1 ($t(9) = 7.801$, $p \leq 0.001$; Cohen's d = 2.467), that is, after novelty was introduced in zone C.

### 3.5. Time spent on contact with tunnels in Zone B (left)

The analysis showed a significant phase by housing setups interaction (Wilks' Lambda; $F(2,27) = 3.360$; $p = 0.007$; $eta^2 = 0.287$), and significant phase differences (Wilks' Lambda; $F(1,27) = 29.788$; $p \leq 0.001$; $eta^2 = 0.781$).

Analysis of variance (ANOVA) was used to analyse the amount of time spent on contact with the tunnels in zone B by individuals from the three housing setups during each phase; it yielded differences between the groups in phase H1/H2 ($F(2,29) = 21.640$, $p \leq 0.001$; $eta^2 = 0.616$); in phase H6/H7 ($F(2,29) = 9.467$, $p = 0.001$; $eta^2 = 0.412$) – Fig. 7. Post hoc analysis using the Tukey HSD test showed that in phase H1/H2, SC rats spent more time on contact with the tunnels than ESC rats ($p \leq 0.001$; $M_{SC} = 96.4$, $SD_{SC} = 15.3$; $M_{ESC} = 58.3$, $SD_{ESC} = 12.5$) and ECC rats ($p \leq 0.001$; $M_{ECC} = 58.7$, $SD_{ECC} = 16.5$; Cohen's d = 2.370). Similarly, in phase H6/H7, SC rats spent more time on contacts with the tunnels than ESC rats ($p = 0.018$; $M_{SC} = 81.4$, $SD_{SC} = 15.1$; $M_{ESC} = 60.2$, $SD_{ESC} = 19.6$; Cohen's d = 1.187) and ECC rats ($p = 0.001$; $M_{ECC} = 50.8$, $SD_{ECC} = 12.9$; Cohen's d = 2.137). No differences between the groups were observed in T1, that is, after novelty was introduced in zone C.

A paired samples Student's *t*-test was used to assess the changes in the amount of time spent by animals from different housing conditions on exploration of the tunnels in zone B between individual phases. In ESC rats, a statistically significant decrease in the exploration time was observed in T1 ($t(9) = 5.225$, $p = 0.001$; Cohen's d = 1.652), that is, immediately after the introduction of novelty in zone C. In SC animals, however, there was a marked decrease in phase H6/H7 ($t(9) = 2.519$,

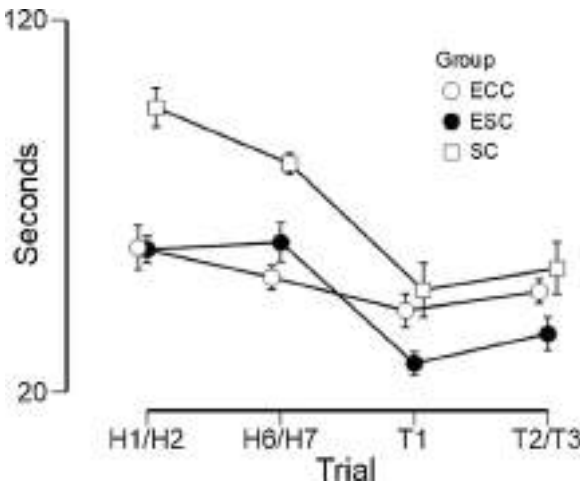


**Fig. 7.** Time (s) spent by rats on contact with objects in the left zone of the experimental chamber. ECC - Enriched changing conditions; ESC - Enriched stable conditions; SC - Standard conditions.

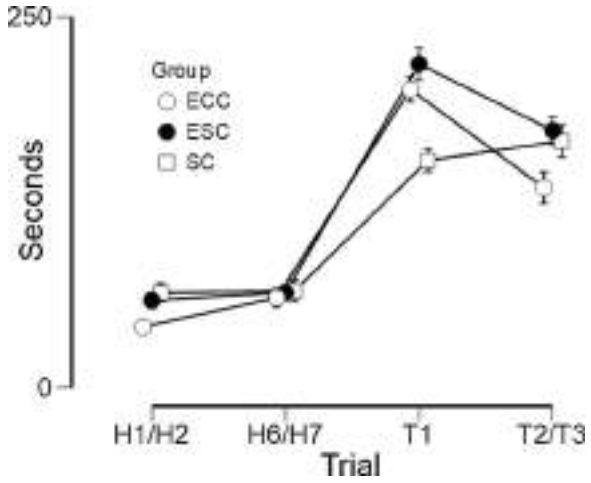


**Fig. 8.** Time (s) spent by rats on contact with objects in the right zone of the experimental chamber. ECC - Enriched changing conditions; ESC - Enriched stable conditions; SC - Standard conditions.

p = 0.033; Cohen's d = 0.797), followed by another decrease in exploration time in T1 (t(9) = 4.419, p = 0.002; Cohen's d = 1.397). IN ECC rats, no significant changes were observed between the individual phases of the experiment (p > 0.05).

### 3.6. Time spent on contact with tunnels in Zone C (right)

The analysis showed a significant phase by housing setups interaction (Wilks' Lambda; F(2,27) = 4.498; p = 0.001; $eta^2$ = 0.351), and significant phase differences (Wilks' Lambda; F(1,27) = 198.972; p ≤ 0.001; $eta^2$ = 0.960).

Analysis of variance (ANOVA) was used to compare the amount of time spent on contact with the tunnels in zone C by individuals from the three housing setups during each phase; it yielded differences between the groups in first habituation phase H1/H2 (F(2,29) = 5.883, p = 0.008; $eta^2$ = 0.304), and T1 (F(2,29) = 14.458, p ≤ 0.001; $eta^2$ = 0.517), that is, immediately after the introduction of novelty – Fig. 8. Post hoc analysis using the Tukey HSD test showed that in the habituation phase H1/H2, SC rats spent more time on contact with the tunnels than ECC rats (p = 0.008; $M_{SC}$ = 64.0, $SD_{SC}$ = 18.4; $M_{ECC}$ = 40.9, $SD_{ECC}$ = 11.3; Cohen's d = 1.542), and ESC rats spent more time on contact with the tunnels than their ECC counterparts (p = 0.045; $M_{ESC}$ = 58.8, $SD_{ESC}$ = 16.8; Cohen's d = 1.187). However, in T1 (after the introduction of novelty), SC rats spent less time on contact with the tunnels than ECC rats (p = 0.002; $M_{SC}$ = 153.2, $SD_{SC}$ = 23.5; $M_{ECC}$ = 201.3, $SD_{ECC}$ = 26.9) and ESC rats (p ≤ 0.001; $M_{ESC}$ = 218.3, $SD_{ESC}$ = 32.9; Cohen's d = 0.907).

A paired samples Student's *t*-test was used to assess the changes in the amount of time spent by animals from different housing conditions on exploration of the tunnels in zone C between individual phases. In ECC rats, there was an increase in the amount of time spent on contact with the tunnels in phase H6/H7 (t(9) = -3.551, p = 0.006; Cohen's d = 1.123), followed by a sharp decrease in the first phase after the introduction of novelty, that is, T1 (t(9) = -15.002, p ≤ 0.001; Cohen's d = 4.744), which was subsequently followed by a decrease in phase T2/T3 (t(9) = 4.559, p = 0.001; Cohen's d = 1.442). In ESC rats, on the other hand, the statistically significant aspect was the increase in the frequency of contacts with the tunnels only in T1 (t(9) = -13.437, p ≤ 0.001; Cohen's d = 4.249), and the subsequent decrease in phase T2/T3 (t(9) = 2.836, p = 0.020; Cohen's d = 0.897). In SC rats, there was an increase in T1 (t(9) = -8.826, p ≤ 0.001; Cohen's d = 2.791).

Cohen's d was used to estimate the differences in effect sizes for contacts with the tunnels in zone C between the last habituation phase (H6/H7) and the first test phase (T1). The effect size was smaller in the control group (d = 4.57; r = -0.92; $M_{SC\_H7}$ = 65.2, $SD_{SC\_H7}$ = 13.6; $M_{SC\_T1}$ = 153.2, $SD_{SC\_T1}$ = 23.5) than in the ECC group (d = 6.45; r = -0.95; $M_{ECC\_H7}$ = 56.2, $SD_{ECC\_H7}$ = 16.9; $M_{ECC\_T1}$ = 201.3, $SD_{ECC\_T1}$ = 29.9) and the ESC group (d = 6.75; r = -0.96; $M_{ESC\_H7}$ = 50.1, $SD_{ESC\_H7}$ = 12.5; $M_{ESC\_T1}$ = 218.3, $SD_{ESC\_T1}$ = 32.9). No differences in effect size for contacts with the tunnels were observed between the ECC and ESC groups.

### 3.7. Grooming

Time spent on grooming in each test phase was measured.

The analysis showed no significant phase by housing setups interaction (Wilks' Lambda; p = 0.738), but a significant main factor effect for the phase (Wilks' Lambda - F(1,27) = 5.336, p = 0.006; $eta^2$ = 0.390).

A visual data analysis revealed that in laboratory rats, the amount of time spent on grooming was low in all the groups under study throughout the experiment; the values fell within the range of 3.4–32.3 seconds.

A paired samples Student's *t*-test was used to assess the changes in the amount of time spent on grooming by all the animals tested between individual trials. A significant decrease in grooming activities was only observed in trial T2/T3 (t(28) = 2.293, p = 0.034; Cohen's d = 0.419).

## 4. Discussion

The analysis of exploratory behaviour revealed that at the beginning of the experiment, rats maintained in the standard environment were more active in the tunnel zones than their enriched housed counterparts. They spent more time exploring the objects and less time in the starting box and the central zone. This may suggest that rats from the control group deprived of the possibility of interacting with the objects in their home environment exhibited a higher propensity for interactions with a new environment (cf. Fernandez-Teruel et al., 1997; Tanas et al., 2015; Makowska and Weary, 2016). It is also possible that the adaptation to the test environment occurred more slowly in the individuals from this group, and that they spend more time familiarising themselves with the surroundings (Matzel and Sauce, 2017). Nevertheless, at the end of the habituation period, activity levels were almost equal in all study groups, which may point to a similar level of habituation to the experimental arena. After the introduction of novelty to one of the zones, all study groups showed markedly higher exploration of the new objects. Controls, however, spent the least time in the altered zone and the least time exploring the new objects. In addition, the enriched housed rats quickly habituated to the changes, and the level of exploration of the new objects fell in subsequent trials, while the control rats maintained their high exploration levels in that zone throughout subsequent test trials. This suggests an impact of environmental experience on the learning process. Habituation is, after all, a form of learning, which means that slow habituation of response to change in a familiar environment indicates slower modification of behaviour. These findings are in accordance with the results of other studies in which was found that environmental enrichment accelerated habituation to novelty (Schrijver et al., 2012; Zimmermann et al., 2001). A high level of exploration after the introduction of novelty in the control group may confirm the above assumption that this group was characterised by a slower pace of adaptation to change.

Specific differences were also observed in the level of exploratory behaviour in rats maintained in the two enriched setups. In rats from the changeable environment, no significant changes were recorded in the amount of time spent exploring the objects in the zones which remained unaltered throughout the experiment. In rats housed in the stable environment, there was a marked decrease in exploration of objects in the unaltered zone after novelty was introduced in the other one. However, this had no impact on the increase in the amount of time

spent by both groups on exploring the new objects, and the effect size of this increase was comparable in both groups as well.

The lack of specific differences between rats housed in enriched stable and changing environments may suggest that the enriched stable conditions, despite their lack of changeability, were complex and stimulating enough to enable the rats to manipulate the level of environmental complexity and diversity on their own. This possibility of environmental manipulation allowed them to regulate the level of incoming environmental stimulation. Moreover, the animals' locomotor activity in the home environment may have been a significant source of environmental variability for the other individuals. In addition, it may be concluded that in laboratory rats, a highly and constantly changing environment did not change the anxiety level as observed at the behavioural level. This is in line with a widely acknowledged finding (Manosevitz and Pryor, 1975), which indicates that cage dimensions alone are the crucial aspects of environmental enrichment. Those results may be compared with the results of a study conducted on mice (Bailoo et al., 2018). A comparative analysis of behaviour in animals kept in conditions characterised by different enrichment levels showed that the highest welfare rate (lowest level of stress and chronic fear) was observed for mice housed in a highly enriched environment in which additionally a change was introduced every week. Nevertheless, it is difficult to draw any unambiguous conclusions about the specific impact of the change on the animals' welfare. In contrast to the other environmental settings used in the study, the environmental changeability was combined with the largest range of enriching elements, as well as a significantly larger cage available space. It is therefore possible that, as mentioned above, the enlarged cage space was the most significant element in that study as well.

The absence of differences in the level of exploration between the animals from the stable and changeable environments may also be linked to the relatively quick extinguishment of behaviours reinforced by intrinsic reinforcement and the quick habituation of curiosity in conditions of constant change (Tarou and Bashaw, 2007). This assumption may be supported by the fact that the animals were housed in an enriched environment for a long time, while the changes to the environment, albeit constant, were of similar nature. It may be concluded, therefore, that in a setting characterised by long-lasting environmental enrichment, the changeability of the environment plays no major role, at least with respect to exploratory behaviours, general activity level and the pace of habituation to the change encountered in that environment.

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PLOS ONE

RESEARCH ARTICLE

# Rat's response to a novelty and increased complexity of the environment resulting from the introduction of movable vs. stationary objects in the free exploration test

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## Abstract

Most animals, including rats, show a preference for more complex environments. This is demonstrated particularly well when complexity increases due to the addition of new elements to the environment. The aim of the study was to investigate the reaction to novelty, understood as a change in environmental properties that involve both changes in complexity and controllability. Controllability may allow for dealing with challenges of an environment of low predictability in a way that the animal's own activity reduces the uncertainty of environmental events. In our study, the animals underwent a spontaneous exploration test in low-stress conditions. After a period of habituation to the experimental arena, additional stationary (increased complexity) and/or movable (increased complexity and controllability) tunnels were introduced, and the reaction of the rats to the novel objects was measured. The results of the study confirmed that an increase in the complexity of the environment through the addition of objects triggers a more intensive exploratory activity in rats. However, an increased spatial complexity combined with the movability of the novel objects seems to result in increased caution towards the novelty after an initial inspection of the changed objects. It suggests that the complexity of the novelty may trigger both neophilia and neophobia depending on the level of the predictability of the novel environment and that the movability of newly introduced objects is not independent of other parameters of the environment.

## 1. Introduction

Complexity is a key aspect of any environment that a given organism encounters [1]. Environmental complexity can be related to the number of elements present in the environment, the number of available sources of stimulation, or relations between the different objects in the environment [2]. According to Godfrey-Smith [3], the best definition of complexity is very simple–complexity equals lack of homogeneity; complexity equals heterogeneity.

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Under natural conditions, an environment can be predictable (e.g. day-night cycle) or unpredictable (e.g., predators, weather changes, human disturbance) [4]. Managing environmental unpredictability is one of the biggest challenges animals encounter throughout their lives [5]. When an unexpected event occurs in the surroundings, an animal must respond to it quickly by engaging in appropriate behaviors, drawing on its previous experience [5]. Berlyne [6] distinguishes the following properties of stimuli present in the environment: novelty, change, complexity and 'surprisingness', which initiate and sustain exploratory behaviors.

In an unpredictable environment, the elements present can change, and their properties can change at any time. In a laboratory setting, we are able to control almost every aspect of the environment. Therefore, a complex environment can be changeable or unchangeable, and every aspect of emergent novelty is controlled [7]. Novelty appearing in the environment plays an important role in shaping animal behavior. The response to a novel stimulus appearing in the environment is related to the animal's genetic background [8] and its prior experience [9]. The relationship between brain memory systems and neotic preferences involves novelty detection in which current experiences are compared with encoded information about past events [10–12]. A discrepancy between the stored data and the current event is detected as novelty and addressed with specific behaviour [13]. The reaction to novelty may elicit neophobia (avoidance) and neophilia (approach). The former protects the animal from danger, while the latter is associated with gathering information from the environment [14]. The extent of neophobia and neophilia is related to the degree of novelty generated by the stimuli present in the environment [15].

The discussion about environmental complexity may benefit from the inclusion of the term 'affordances', coined by J.J. Gibson in the 1970's [16]. At an operational level, affordances may be compared to a range of 'options' an environment offers its inhabitants; a range of available behaviors it provides [17]. It must be borne in mind, however, that the ability to interact with an object is closely linked to an organism's perceptual abilities and the complexity of its nervous system. Depending on the level of its biological complexity, an organism can process and search for information at different levels of sensory, logical, and content-oriented complexity ([18], p. 35). In this context, 'information' means any event that plays a role in the animal's behavioral regulation by attributing meaning to an environment-derived stimulus–provided that such information can be deciphered by the animal's sensory apparatus ([18], p. 35). Many stimuli present in the environment may be available to the animal's perceptual apparatus, but if the animal cannot use these stimuli as behavior-regulating factors, what occurs is stimulation and not information ([18], p. 35). In order to take advantage of what an environment has to offer, an organism must have the capacity to process information provided by the environmental stimuli it receives [19].

Uncertainty is yet another aspect of environmental complexity that every organism encounters. Uncertainty is a psychological state of the organism that occurs when it is faced with events occurring in the environment, the consequences of which are difficult to predict [20]. At every level of biological complexity, organisms must manage uncertainty linked to the signals or stimuli they receive from their environments and the possible consequences of actions triggered by those signals [21]. Based on their previous experience, individuals form expectations as to future events. These expectations play a major role in the reaction to new stimuli and their interpretation [21]. The ability to manage unfamiliar and novel situations is of fundamental importance in complex environments. Some researchers believe that the need to manage environmental uncertainty has not been given sufficient consideration in the discussion on what determines animal behavior [5]. Environmental uncertainty undoubtedly involves predation risk, among other things. Predation is also the main selection factor in the evolutionary processes shaping animal morphology and behavior (e.g., [22]). The risk of falling prey to

predators may also vary seasonally, and in some cases, it may change from one minute to the next, so animals must exhibit behavioral flexibility in order to adjust their predator-avoidance strategies to the ever-changing situation [22].

Studies show that rats prefer more complex environments [23, 24]. Moreover, complex environments help reduce anxiety and increase the rat's activity [25], stimulating novelty-seeking behaviors [26]. The results of experiments suggest that rats prefer environments where the level of complexity is higher than the level they previously encountered [27, 28]. In our previous experiments, environmental complexity was manipulated by increasing the number of elements (addition of tunnels) or decreasing the number of elements (removal of tunnels) in the test environment. Increasing the number of tunnels in the test phase triggered a significant change in the animals' behavior. The rats spent more time near the new tunnels, sniffed them and came into contact with them, climbed on top of them and hid inside them. Our results suggest that rats exhibit positive reactions to new objects which do not trigger a stress response [29–33]. An animal confronted with a new stimulus in low-stress conditions is likely to engage in an activity involving approaching the source of change and exploring the novel element(s) in the environment [18 (p. 75)].

In laboratory conditions, we are able to manipulate environmental complexity. One of our previous studies focused on the impact of affordances in an enriched living environment on the animals' behavior in an exploration test [7]. It turned out that the rats kept in standard laboratory cages (with no enrichment) exhibited a higher level of exploratory behavior during the test than the rats kept in enriched environments. This suggests that rats previously kept in standard laboratory cages needed more time to familiarize themselves with the test environment [34]. It is also possible that depriving them of the possibility of interacting with various objects in the home cage resulted in an increased likelihood that they would explore new objects in the test arena [35–37]. Although rats kept in an enriched environment are more active and exhibit more exploratory behaviors while they stay in these kinds of conditions [e.g., 38, 39], it seems that rats who are deprived of such opportunities in their living cages compensate for their need for exploration with more heightened exploration when they are given a chance.

Studies suggest that another important feature of an animal's environment is the possibility of controlling it. Controllability is defined as the ability of animals to alter aspects of their environment (e.g., by moving or breaking items in their surroundings)—[40]. Captive animals will use controllable items more frequently than items they cannot control [41], and control over the environment may attenuate physiological stress responses [42, 43]. Sambrook & Buchanan-Smith [44] even suggest that controllability of the environment is more important than complexity when considering environmental enrichment for captive animals. Additionally, controllability may allow for dealing with challenges of an environment of low predictability in a way that the animal's own activity reduces the uncertainty of environmental events (cf. [5]). Wild rats (*Rattus norvegicus)* inhabit almost all land environments. They adapt easily to the surrounding conditions thanks to their biological predispositions, omnivorous diet and behavioral flexibility [45]. Their living environment is characterized by a high degree of changeability, unpredictability and, therefore, uncertainty. During exploration, rats gather information about the surrounding environment [46]. However, the exploratory behaviors they exhibit are not homogenous. Berlyne [47] introduced the term 'diversive exploration', which is aimed at gathering information about the surroundings, and 'specific exploration', which involves examining new objects by the animal. Renner and Seltzer [48] divided rats' exploratory behaviors into general exploration and examination of novel objects. As in Berlyne [47], the term 'exploration' was used here in the general sense of describing a range of behaviors including moving from one place to another and sniffing, while the term 'investigation'

(i.e. 'specific exploration') was used to denote interactions with specific aspects of the environment such as objects that are sources of stimulation.

Our present work is a continuation of a series of studies on the various aspects of environmental novelty in rat behavior regulation [27, 28]. This study is aimed at the analysis of the reaction to novelty, understood as a change in environmental properties that involve both change in complexity and controllability (the presence of movable and unmovable objects). Therefore, the novelty did not entail increasing or decreasing environmental complexity by adding or removing elements only but also involved providing rats with the possibility of manipulating movable objects, thereby allowing them to regulate stimulation originating in the environment.

We expected that novelty in the form of increased environmental complexity and change in the properties of the environment would have an impact on the animals' behavior, resulting in 'specific exploration' [47]. Movable tunnels introduce greater complexity and controllability on the environment but also account for greater uncertainty. These factors would likely motivate rats to explore more extensively than in the case of the stationary tunnels which only introduce more complexity.

Additionally, movable objects were expected to provide animals with the possibility to control their environment by manipulating the objects. An increase in environmental complexity (adding tunnels and replacing stationary tunnels with movable ones) provides stimuli bearing the features identified by Berlyne [6]. It could therefore be assumed that this type of experimental manipulation would expose the animals to challenges posed by the need to function in a complex environment. Animals can provide themselves with sensory reinforcement different from the reinforcement they receive when interacting with stationary elements. Encountering movable objects and the resulting stimulation due to the animal's own activity should have a rewarding value (cf. [49]).

The experiment was carried out in low-stress conditions, with the new objects being a source of positive stimulation, which allowed us to assume the rewarding value of novelty thus introduced. Low-stress conditions were secured by a long habituation phase followed by the introduction of low-intensity novelty [50]. During the experiment, the rats were free to explore the experimental arena and were allowed to enter the transporter (a safe, familiar environment—cf. [51]) at all times. The low-stress experimental conditions enable animals to express a variety of behavioral species-specific repertoire.

## 2. Materials and methods

### Animals

The sample consisted of 35 male Lister Hooded rats. The rats were bred and housed in the vivarium of the Institute of Psychology, Polish Academy of Sciences, Warsaw, Poland. At the beginning of the study, the rats were approx. 90 days old and weighed approx. 350g.

The rats were housed in groups of 3–4 in Tecniplast© Eurostandard Type IV cages (610mm×435mm×215mm) with dust-free softwood granules Tierwohl Super© as bedding. They had ad libitum access to water and standard laboratory fodder (Labofeed H, WP Morawski, Kcynia, Poland). The day/night cycle was set at 12/12h (lights-on at 8.00 a.m.). The temperature was maintained at a constant 21-23°C, and humidity at 45–60%. Prior to the experiment, the cages were cleaned once a week. However, in order to ensure that the experimental procedure was not disturbed, the cages in which the test animals were kept were cleaned just before the start of the behavioral test and again after the test was completed. The study took place daily between 11 a.m. and 2 p.m. The rats were always tested in the same order.

All the rats were housed, bred, and taken care of in accordance with the Regulation of the Polish Minister for Agriculture and Rural Development of 14 December 2016 on laboratory animal care. The experimental procedures had been approved by the First Local Committee for Ethics in Animal Experimentation in Warsaw, Poland, permit #1115/2020.

## Procedure

The experiment followed the protocol for conducting tests on rats in low-stress conditions [50]. Low-stress conditions were ensured by prolonged habituation prior to the start of the testing phase, the introduction of low-intensity novelty, and conducting the study in the dark. The rats were allowed to enter the transporter during the entire study. The experimental apparatus was the same as the apparatus used in our previous studies [7, 27, 50]. The experimental manipulation consisted in increasing the number of tunnels or changing the properties of the tunnels, depending on the experimental group. Therefore, the introduction of novelty in the form of movable tunnels did not involve placing completely new objects in the experimental arena but rather consisted in changing the properties of elements with which the animals had been familiarized before.

The experimental chamber (Fig 1) was a box measuring 800mm×600mm×800mm. A detailed description has been provided elsewhere [27, 50]; therefore, we show only a general outline of the device. The chamber was divided into three zones: A, B, and C, with two walls running perpendicularly to its longer side. The partition walls between the zones had triangular openings (120mm×140mm) at the bottom, which enabled free movement between the

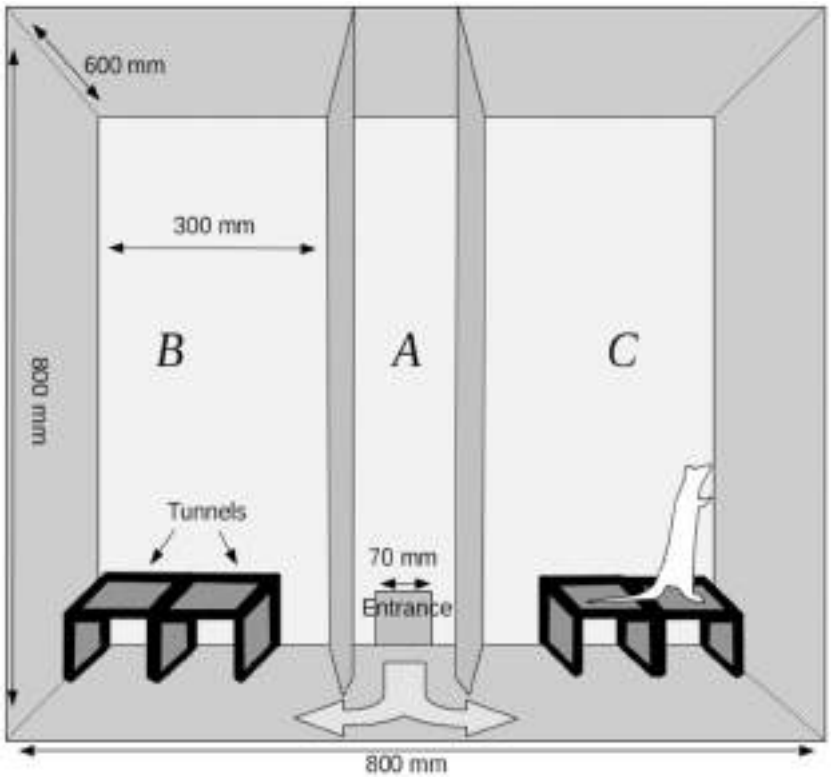


**Fig 1. A schematic view of the experimental chamber.** A transporter was set up at the entrance. The rat had free access to the transporter at all times. In Zone B the arrangement of the tunnels was the same all the time, in Zone C, on the test days (T1, T2, T3), it was changed depending on the test group.

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chamber parts. There was a hole curved in the back wall of the chamber, which served as an entrance for animals going from the transporting device into the chamber. The front of the chamber was made of transparent plexiglass, and it could be lifted to obtain full access to the experimental arena. The entire chamber was covered with a layer of washable varnish. There were tunnels (200mm×120mm×80mm) placed in zones B and C made of hardwood covered with washable paint. In contrast to the most frequently used two-dimensional experimental settings, these tunnels provide a complex three-dimensional environment. The central zone (A) was left empty.

At the start of each trial, a small cylindrical cage (the 'transporter'– 60mm in diameter with doors 120mm high and 100mm wide) with the tested animal inside was placed by the entrance to zone A. The entrance door was then lifted, and it was left open until the end of the trial. The animal was free to stay in the transporter or leave it to explore the chamber. The first seven trials were habituation trials during which the apparatus was arranged in the same way: in zones, B and C, the setting of the objects was the same (two stationary tunnels in each of the two chambers). There was no access to food or water in the testing apparatus.

The introduction of novelty took place between trials 7 and 8. The three subsequent trials were conducted with the chamber in this new arrangement (Fig 2). Each trial was 7 minutes long and was conducted for each animal once a day.

In the habituation phase, two stationary tunnels (200mm×120mm×80mm) were placed in each of zones B and C and arranged in the same manner (Fig 1). Test trials differed with regard to the configuration of the tunnels that were placed in the experimental chamber.

Setting 1 - (Mov) Movement of familiar objects in the experimental box. On the first experimental day (trial 8), movable (see-saw-like) tunnels were placed in zone C (Fig 2). These movable tunnels replaced stationary tunnels from the habituation phase. The arrangement of the tunnels in zone B remained unchanged. The Mov group consisted of 10 rats.

Setting 2 - (MovAdd) Addition of a novel movable object in the experimental box. On the first experimental day (trial 8), two supplementary tunnels were placed on top of the tunnels from the habituation phase in zone C (Fig 2). The tunnel at the very top was a movable (see-saw-like) tunnel. The arrangement of the tunnels in zone B remained unchanged. The MovAdd group consisted of 10 rats.

Setting 3 - (Add) Addition of novel stationary objects in the experimental box. On the first test day (trial 8), two tunnels placed on top of one another were added to the two tunnels in zone C. The tunnels in zone B remained in the same configuration as before. The Add group consisted of 15 rats.

To avoid the deceptive effect of lateralization or visual-auditory cues, the novelty was implemented in the right zone (as described above—zone C) for half of the test rats and in the left zone (zone B) for the remaining half (mirror image of Fig 2).

## Data processing and statistical analyses

The study was recorded in the dark with the use of a night vision camera (BSC-THC3400IR, HD-CVI 4MPX IR:30M) placed approx. 1.5m from the study apparatus. To code the behaviors on the basis of the recorded material, we used BORIS software [52], which made it possible to define selected behaviors and assess their duration and frequency. We scored the behaviors the animals engaged in during the entire experimental trial. Consequently, we were able to assign specific scores to the time of separate bouts of behaviors, their frequency, and the total time an animal spent engaging in a given behavior. The following variables were measured: (1) Time spent in the transporter (excluding the latency to leave the transporter); (2) Time spent in the unchanged zone of the chamber; (3) Time spent in the changed zone of the chamber; (4)

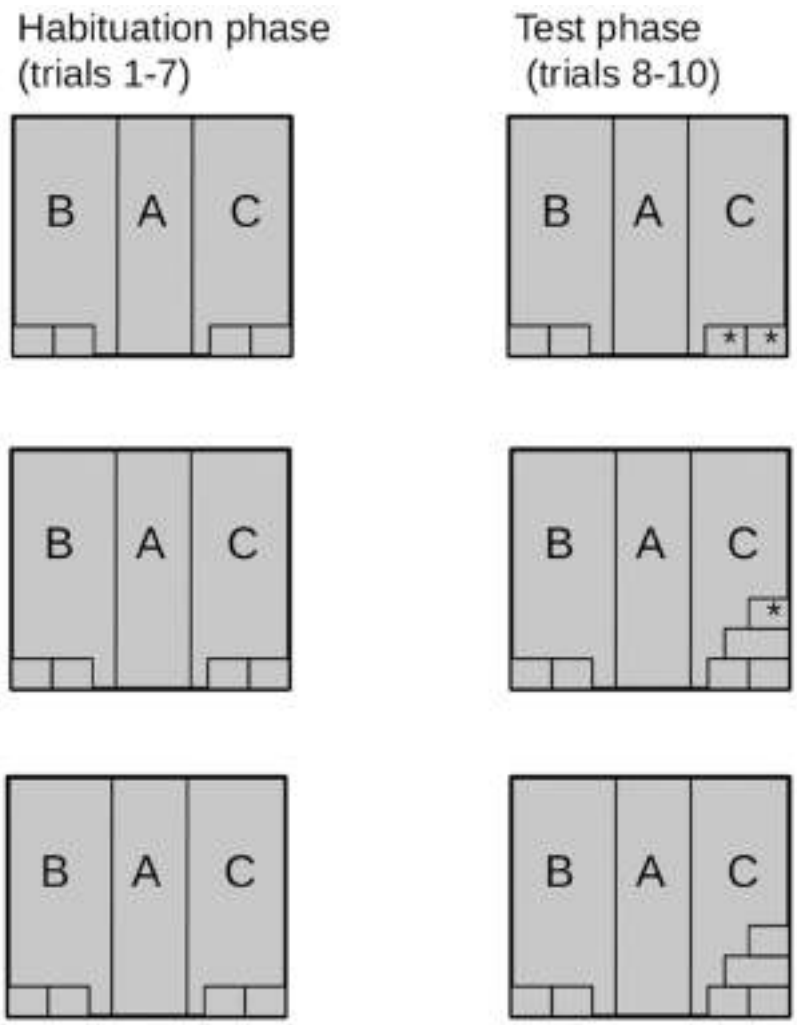


**Fig 2. Arrangement of objects in the experimental chamber in each experimental setting.** Tunnels marked with * were the see-saw-like tunnels. On the left, the setting during habituation is presented. On the right, the setup during the test days is presented: in the Mov group, two stationary tunnels were replaced by two movable tunnels (zone C). In the MovAdd group, tunnels were added, and the tunnel on the sting was movable (zone C). In the Add group, tunnels were added, and there were no movable tunnels.

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Frequency of moving between the zones (left/right/transporter) of the chamber; (5) Time spent on contact with the tunnels in the changed zone of the chamber; (6) Frequency of contact with the tunnels in the unchanged zone of the chamber; (7) Time spent on contact with the tunnels in the unchanged zone of the chamber; and (8) Frequency of contact with the tunnels in the changed zone of the chamber.

To enhance the legibility of the results and tables, the habituation phase has been indicated as H (mean score from habituation trials 5 to 7, which served as a reference value for further analyses), while the test trials have been indicated as T1, T2, and T3, respectively. Novelty (i.e., addition of tunnels or addition and change of properties of tunnels in zone C) was introduced in the first test trial (T1).

We have decided not to present the results of the initial four habituation trials, as they serve only as the habituation phase and not as an element of a comparative analysis of the animals' response to novelty.

The data were analyzed using a General Linear Model procedure (GLM), with repeated measurements (H, T1, T2, T3) as within-subject factors, as well group assignment (Mov, Add, MovAdd) as between-subject factors. PostHoc *t*-tests were carried out subsequently with Bonferroni correction for multiple comparisons. Differences were considered significant for $p \leq 0.05$. Descriptive statistics of all behavioral measurements are presented in Table 1.

**Table 1. Descriptive statistics of all behavioral measurements analyzed in this study.** Group Add—addition of novel tunnels; group MovAdd—addition of movable tunnel; group Move—replacement of familiar tunnel for movable one. H—habituation phase; T1-T2—consecutive trials.

| Group | Add N = 15 | | Mov N = 10 | | MovAdd N = 10 | |
|---|---|---|---|---|---|---|
| **Trials** | **Mean** | **Std dev** | **Mean** | **Std Dev** | **Mean** | **Std Dev** |
| **Time spent in the transporter** | | | | | | |
| H | 62,588 | 18,430 | 52,633 | 12,385 | 43,900 | 20,344 |
| T1 | 37,588 | 20,087 | 44,000 | 15,420 | 28,900 | 14,177 |
| T2 | 42,059 | 26,541 | 48,200 | 16,491 | 21,500 | 8,923 |
| T3 | 38,294 | 19,274 | 39,800 | 16,199 | 38,600 | 25,726 |
| **Time spent in the unchanged zone of the chamber** | | | | | | |
| H | 125,588 | 19,162 | 138,200 | 18,887 | 137,500 | 42,023 |
| T1 | 69,118 | 20,624 | 106,800 | 18,152 | 78,500 | 26,983 |
| T2 | 97,118 | 38,498 | 118,700 | 37,547 | 103,900 | 32,285 |
| T3 | 86,235 | 34,523 | 127,000 | 46,671 | 127,100 | 34,598 |
| **Time spent in the changed zone of chamber** | | | | | | |
| H | 126,607 | 23,890 | 101,533 | 24,014 | 109,333 | 25,735 |
| T1 | 217,059 | 36,689 | 137,900 | 28,838 | 222,200 | 48,371 |
| T2 | 208,059 | 65,480 | 140,600 | 44,488 | 187,200 | 51,963 |
| T3 | 224,824 | 47,870 | 142,700 | 35,214 | 164,600 | 42,380 |
| **Frequency of moving between the chamber zones (left/right/transporter)** | | | | | | |
| H | 14,569 | 2,021 | 22,967 | 3,245 | 20,633 | 3,008 |
| T1 | 12,824 | 2,580 | 20,100 | 5,131 | 17,900 | 3,213 |
| T2 | 13,176 | 4,348 | 21,900 | 2,846 | 17,800 | 2,394 |
| T3 | 13,471 | 3,064 | 22,300 | 4,762 | 19,900 | 3,604 |
| **Time spent on contact with the tunnels in the changed zone of the chamber** | | | | | | |
| H | 76,059 | 19,084 | 60,333 | 16,555 | 66,333 | 20,292 |
| T1 | 167,765 | 34,485 | 96,200 | 32,495 | 181,000 | 50,767 |
| T2 | 160,294 | 56,553 | 92,600 | 40,722 | 144,500 | 48,153 |
| T3 | 182,000 | 43,742 | 89,600 | 34,900 | 122,200 | 32,775 |
| **Frequency of contact with the tunnels in the unchanged zone of the chamber** | | | | | | |
| H | 6,568 | 1,224 | 9,467 | 1,229 | 9,600 | 3,813 |
| T1 | 4,882 | 1,933 | 8,500 | 2,321 | 6,700 | 2,584 |
| T2 | 5,118 | 1,900 | 8,000 | 2,625 | 7,600 | 1,647 |
| T3 | 4,706 | 1,490 | 8,500 | 1,080 | 8,400 | 2,119 |
| **Time spent on contact with the tunnels in the unchanged zone of the chamber** | | | | | | |
| H | 78,098 | 16,652 | 79,333 | 11,389 | 82,567 | 29,019 |
| T1 | 47,706 | 25,544 | 69,000 | 15,195 | 41,600 | 15,778 |
| T2 | 53,118 | 18,927 | 74,100 | 23,965 | 68,100 | 27,201 |
| T3 | 51,235 | 23,212 | 80,800 | 31,094 | 79,400 | 27,925 |
| **Frequency of contact with the tunnels in the changed zone of the chamber** | | | | | | |
| H | 6,235 | 1,678 | 7,900 | 1,899 | 7,900 | 2,091 |
| T1 | 7,529 | 1,972 | 9,900 | 2,079 | 10,800 | 2,616 |
| T2 | 6,647 | 2,499 | 9,200 | 1,874 | 9,300 | 1,767 |
| T3 | 7,235 | 2,251 | 8,300 | 4,191 | 8,700 | 1,703 |

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## 3. Results

### Time spent in the transporter

The analysis showed the main effect of the trial: $F(3,96) = 5.165$, $p = 0.002$, $Eta^2 = 0.139$ (Wilks' Lambda) and a main effect of group: $F(2,32) = 6.262$, $p = 0.005$, $Eta^2 = 0.281$. There was no interaction effect of trial and group.

A post hoc analysis showed a significant **decrease** in the time spent in the transporter **in the first test trial** compared to the habituation phase ($t = 3.058$; $p = 0.017$, Cohen's $d = 0.517$), a significant **decrease** in time spent in the transporter **in the second test trial** compared to the habituation phase ($t = 2.913$; $p = 0.004$, Cohen's $d = 0.598$) and a significant decrease **in the third test trial** compared to the habituation test ($t = 2.913$; $p = 0.027$, Cohen's $d = 0.492$). There were no changes in the time spent in the transporter between the test trials.

There were also differences in the time spent in the transporter between the groups. Rats from the Add group spent more time in the transporter compared to the rats from the MovAdd ($t = 3.491$; $p = 0.004$, Cohen's $d = 0.590$). There were no differences between the group Mov and Add group ($p = 0.188$) and group Mov and group MovAdd (p = 0.490).

### Time spent in the unchanged zone of the chamber

The analysis showed a significant trial by group interaction: $F(3, 96) = 2.804$, $p<0.015$, $Eta^2 = 0.149$ (Wilks' Lambda) and the main effect of the trial: $F(3, 96) = 12.722$, $p<0.001$, $Eta^2 = 0.0.284$ (Wilks' Lambda).

For the MovAdd rats, a post hoc analysis showed a significant **decrease** in the time spent in the unchanged zone of the chamber **in the first trial** compared to the habituation phase ($t = 5.283$; $p<0.001$, Cohen's $d = 1.670$) and a significant decrease **in the third trial** compared to the first trail ($t = 4.352$; $p<0.002$, Cohen's $d = 1.566$). No differences were observed between the trials in the Mov (H-T1, $p = 0.395$; T1-T2, $p = 1.000$; T2-T3, $p = 1.000$) and Add groups (H-T1, $p = 1.000$; T1-T2, $p = 1.000$; T2-T3, $p = 1.000$).

### Time spent in the changed zone of the chamber

The analysis showed a significant trial by group interaction: $F(3, 96) = 4.757$, $p<0.001$, $Eta^2 = 0.229$ (Wilks' Lambda) and the main effect of trial: $F(3, 96) = 36.563$, $p<0.001$, $Eta^2 = 0.533$ (Wilks' Lambda)–Fig 3.

For the Add rats, a post hoc analysis showed a significant **increase** in the time spent in the changed zone of the chamber **in the first test trial** compared to the habituation phase ($t = 6.146$; $p<0.001$, Cohen's $d = 1.701$), a significant **increase in the second test trial** compared to the habituation phase ($t = 4.623$; $p = 0.001$, Cohen's $d = 1.399$) and a significant **increase in the third test trial** compared to the habituation phase ($t = 7.526$; $p = 0.001$, Cohen's $d = 2.239$). There were no differences between the test trials.

For the MovAdd rats, a post hoc analysis showed a significant **increase** in the time spent in the changed zone of the chamber **in the first test trial** compared to the habituation phase ($t = 8.028$; $p<0.001$, Cohen's $d = 2.913$), a significant **increase in the second test trial** compared to the habituation phase ($t = 5.576$; $p = 0.001$, Cohen's $d = 1.899$) and a significant **increase in the third test trial** compared to the habituation phase ($t = 3.957$; $p = 0.010$, Cohen's $d = 1.576$). There is also a significant **decrease** in the third test trial compared to the first test trial ($t = 4.124$; $p = 0.005$, Cohen's $d = 1.266$). No differences were observed in the Mov group (H-T1, $p = 0.705$; T1-T2, $p = 1.000$; T2-T3, $p = 1.000$).

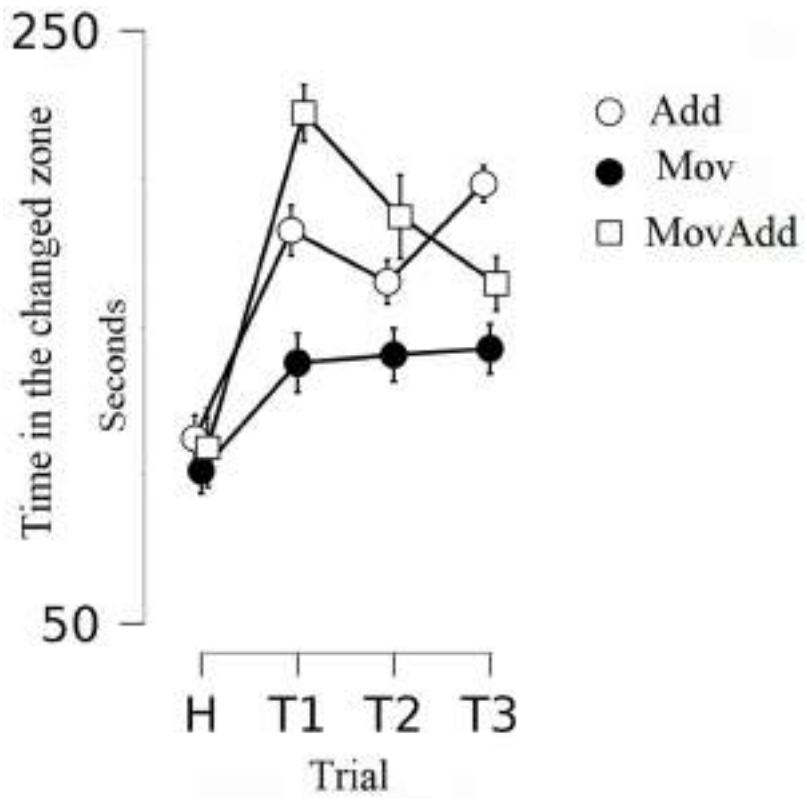


**Fig 3. Mean time spent by rats in the changed zone of the chamber.** Group Add: addition of novel stationary objects in the experimental box, group Mov: movement of familiar objects in the experimental box, group MovAdd: addition of a novel movable object in the experimental box. Error bars represent standard error (SE).

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## Frequency of moving between the chamber zones (left/right/transporter)

The analysis showed a significant main effect of trial: $F(3,96) = 5.533$; $p = 0.002$; $Eta^2 = 0.147$ (Wilks' Lambda) and a main effect of group: $F(2,32) = 3.603$, $p<0.039$, $Eta^2 = 0.184$, but no interactive effect of trial by the group.

Post hoc analyses showed a significant **decrease** in the frequency of moving between the zones **in the first test** trial compared to the habituation phase ($t = 3.975$; $p = 0.001$, Cohen's $d = 0.672$).

There was also a difference in the frequency of moving between the chamber zones between the groups. Rats from the MovAdd group ($t = 0.276$; $p = 0.047$, Cohen's $d = 0.047$) showed a higher frequency of moving between the chamber zones than rats from the Add group.

## Time spent on contact with the tunnels in the changed zone of the chamber

The analysis showed a significant trial by group interaction: $F(3, 96) = 6.663$, $p<0.001$, $Eta^2 = 0.294$ (Wilks' Lambda) and the main effect of trial: $F(3, 96) = 46.110$, $p<0.001$, $Eta^2 = 0.590$ (Wilks' Lambda)—Fig 4.

For the Add group, a post hoc analysis showed a significant **increase** in the time spent on contact with the tunnels in the changed zone of the chamber **in the first test trial** compared to the habituation phase ($t = 7.085$; $p<0.001$, Cohen's $d = 1.995$), a significant **increase in the second test trial** compared to the habituation phase ($t = 5.899$; $p = 0.001$, Cohen's $d = 2.278$) and a significant **increase in the third test trial** compared to the habituation phase ($t = 8.856$; $p = 0.001$, Cohen's $d = 2.501$). No differences were observed between the test trials.

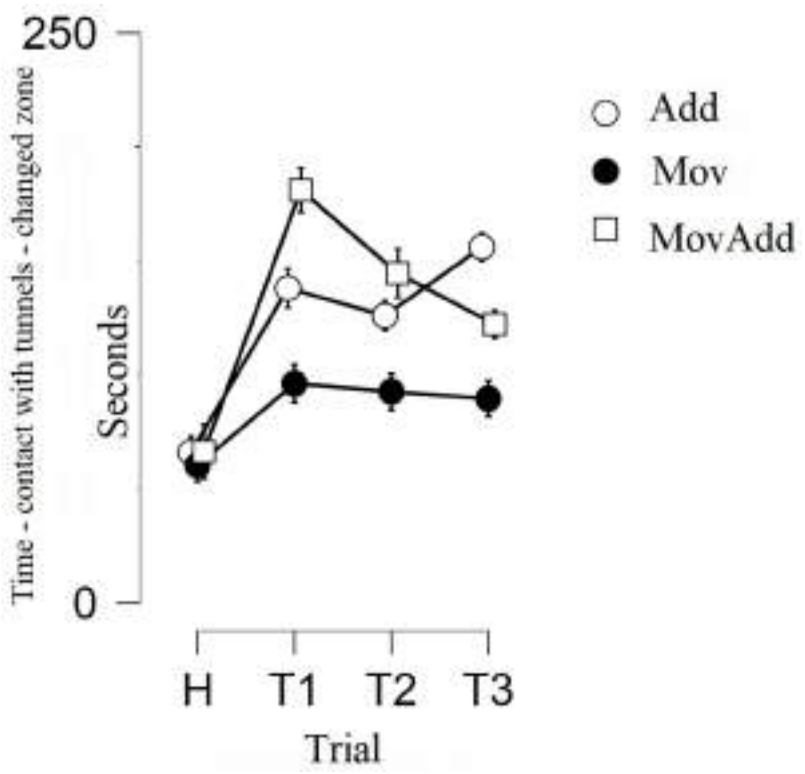


**Fig 4. Mean time spent on the contact with tunnels in the changed zone of the chamber.** Group Add: addition of novel stationary objects in the experimental box, group Mov: movement of familiar objects in the experimental box, group MovAdd: addition of a novel movable object in the experimental box. Error bars represent standard error (SE).

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For the MovAdd group, a post hoc analysis showed a significant **increase** in the time spent on contact with tunnels in the changed zone of the chamber **in the first test trial** compared to the habituation phase ($t = 9.218$; $p<0.001$, Cohen's $d = 2.966$), a significant **increase in the second test trial** compared to the habituation phase ($t = 6.284$; $p = 0.001$, Cohen's $d = 2.115$) and a significant **increase in the third test trial** compared to the habituation phase ($t = 4.491$; $p = 0.010$, Cohen's $d = 2.050$). There was also a significant **decrease** in the third test trial compared to the first test trial ($t = 4.727$; $p = 0.005$, Cohen's $d = 1.376$).

No differences were observed in the Mov group.

## Frequency of contact with the tunnels in the unchanged zone of the chamber

The analysis showed a significant main effect of trial: $F(3,96) = 5.412$; $p = 0.002$; $Eta^2 = 0.145$ (Wilks' Lambda), and a main effect of group: $F(2,32) = 7.230$, $p<0.003$, $Eta^2 = 0.311$. There was no interaction effect of trial and group.

Post hoc analyses showed a significant **decrease** in the frequency of moving between the zones **in the first test** trial compared to the habituation phase ($t = 3.743$; $p = 0.002$, Cohen's $d = 0.633$), a significant **decrease** in the second test trial compared to the habituation phase ($t = 2.887$; $p = 0.029$, Cohen's $d = 0.488$) and a significant **decrease** in the frequency of moving between the zones **in the third trial** compared to the habituation phase ($t = 2.963$; $p = 0.023$, Cohen's $d = 0.501$).

There was also a difference in the frequency of contact with tunnels in the unchanged zone between the groups. Rats from the Move group ($t = 3.569$; $p = 0.003$, Cohen's $d = 0.603$) and from the MovAdd group ($t = 2.631$; $p = 0.039$, Cohen's $d = 0.445$) showed a higher frequency of contact with tunnels than rats from Add group.

## Time spent on contact with the tunnels in the unchanged zone of the chamber

The analysis showed a significant trial by group interaction: $F(3, 96) = 3.014$, $p<0.010$, $Eta^2 = 0.159$ (Wilks' Lambda) and the main effect of trial: $F(3, 96) = 7.562$, $p<0.001$, $Eta^2 = 0.191$ (Wilks' Lambda)—Fig 5.

In the Add and Mov groups, the analyses did not show significant differences in the time spent on contact with the tunnels in the unchanged zone. In the MovAdd group, on the other hand, a post hoc analysis showed a significant **increase** in the time spent on contact with tunnels in the unchanged zone of the chamber **in the first test trial** compared to the habituation phase ($t = 5.039$; $p<0.001$, Cohen's $d = 1.753$) and a significant **decrease** in the third test trial compared to the first test trial ($t = 4.649$ $p = 0.001$, Cohen's $d = 1.666$).

## Frequency of contact with the tunnels in the changed zone of the chamber

The analysis showed a significant main effect of trial: $F(3,96) = 8.979$; $p = 0.001$; $Eta^2 = 0.219$ (Wilks' Lambda), but no interactive effect of trial by the group.

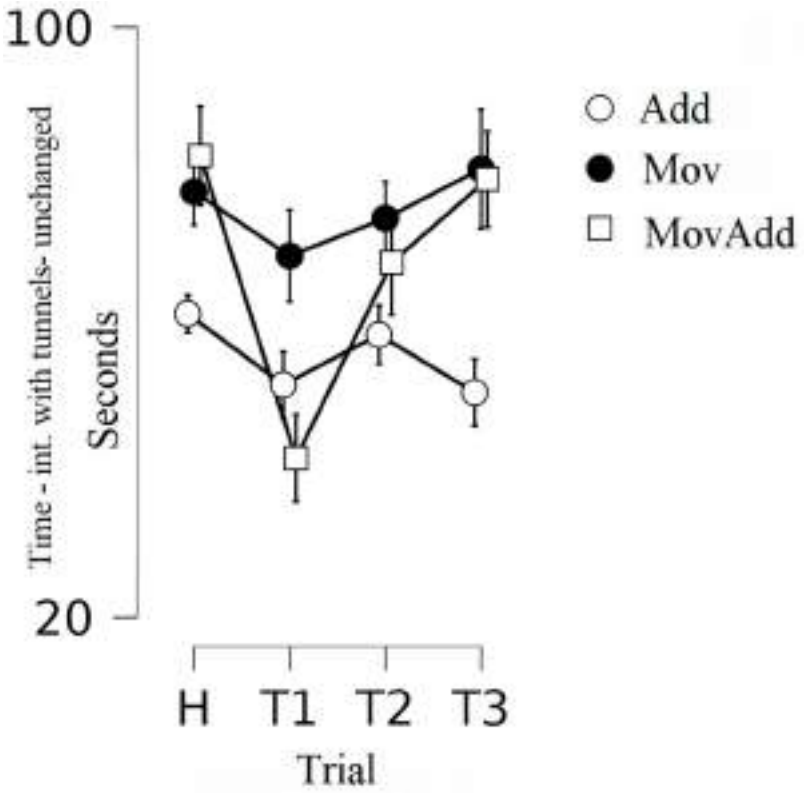


**Fig 5. Mean time spent on contact with the tunnels in the unchanged zone of the chamber.** Group Add: addition of novel stationary objects in the experimental box, group Mov: movement of familiar objects in the experimental box, group MovAdd: addition of a novel movable object in the experimental box. Error bars represent standard error (SE).

https://doi.org/10.1371/journal.pone.0279006.g005

A post hoc analysis showed a significant **increase** in the frequency of contact with the tunnels in the changed zone of the chamber **in the first test trial** compared to the habituation phase ($t = 4.989$; $p<0.001$, Cohen's $d = 0.843$) and a significant **increase in the second test trial** compared to the habituation phase ($t = 3.594$; $p = 0.003$, Cohen's $d = 0.608$).

**Effect size analysis.** In addition to the RM ANOVA analyzes, we conducted an analysis of the size of the obtained effects [27]. We compared all the possible statistical effects according to the obtained Eta partial values using the Kruskal—Wallis ANOVA. The three possible statistical effects (Trial, Group, Trial x Group) were the criteria for grouping, and the number of dependent variables (behavioral measures) was the equivalent of cases.

The results obtained using the Kruskal—Wallis ANOVA (H = 5.15, $df = 2$, $p = 0.07$) showed no differences in the significance of the experimental factors for the variance of the dependent variables (behaviors measured). The lack of a significant result of this analysis indicates that the combination of novelty with object movability produced no additive effect, proving that these environmental properties do not work independently. $Eta^2$ results for the significance of effects are shown in Table 2.

**Summary of the results.** *Mov group*. In the case of the Mov group, two stationary tunnels were replaced with two movable tunnels in the test phase (T1-T3). The number of tunnels did not change. No differences were observed in the amount of time spent in the changed zone or in the amount of time spent on interaction with the changed tunnels.

*MovAdd group*. In the case of the MovAdd group, the number of tunnels was increased in the test phase (T1-T3), and the tunnel placed on top of the other tunnels was a movable one. In

**Table 2. The ranking list of statistically significant effects based on the partial $Eta^2$ values.** The $Eta^2$ values of statistically non-significant effects have been set to "0".

| Dependent | Effect | $Eta^2$ |
|---|---|---|
| Time spent on contact with the tunnels in the changed zone of the chamber | Trial | 0.590 |
| Time spent in the changed zone of the chamber | Trial | 0.533 |
| Time spent in the unchanged zone of the chamber | Group | 0.328 |
| Time spent on contact with the tunnels in the changed zone of the chamber | Group | 0.324 |
| Frequency of contact with the tunnels in the unchanged zone of the chamber | Group | 0.311 |
| Time spent on contact with the tunnels in the changed zone of the chamber | Trial x Group | 0.294 |
| Time spent in the unchanged zone of the chamber | Trial | 0.284 |
| Time spent in the transporter | Group | 0.281 |
| Time spent in the changed zone of the chamber | Group | 0.251 |
| Time spent on contact with the tunnels in the unchanged zone of the chamber | Group | 0.243 |
| Time spent in the changed zone of the chamber | Trial x Group | 0.229 |
| Frequency of contact with the tunnels in the changed zone of the chamber | Trial | 0.219 |
| Time spent on contact with the tunnels in the unchanged zone of the chamber | Trial | 0.191 |
| Frequency of moving between the chamber zones (left/right/transporter) | Group | 0.184 |
| Time spent on contact with the tunnels in the unchanged zone of the chamber | Trial x Group | 0.159 |
| Time spent in the unchanged zone of the chamber | Trial x Group | 0.149 |
| Frequency of moving between the chamber zones (left/right/transporter) | Trial | 0.147 |
| Frequency of contact with the tunnels in the unchanged zone of the chamber | Trial | 0.145 |
| Time spent in the transporter | Trial | 0.139 |
| Time spent in the transporter | Trial x Group | 0 |
| Frequency of moving between the chamber zones (left/right/transporter) | Trial x Group | 0 |
| Frequency of contact with the tunnels in the unchanged zone of the chamber | Trial x Group | 0 |
| Frequency of contact with the tunnels in the changed zone of the chamber | Trial x Group | 0 |
| Frequency of contact with the tunnels in the changed zone of the chamber | Group | 0 |

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this group, differences were observed in the amount of time spent in the changed zone of the chamber. In all test trials (T1-T3), the rats spent more time in the changed zone than they had done during the habituation phase (H). On the second test day (T2), the rats spent more time in the changed zone than on the third test day (T3). Throughout the test phase (T1-T3), the rats spent more time on contact with the tunnels than during the habituation phase (H). On the first test day (T1), they spent more time on contact with the tunnels than on the third day (T3).

*Add group*. For this group, the number of tunnels was increased in the test phase (T1-T3), but no movable tunnels were added. Throughout the test phase (T1-T3), the rats spent more time in the changed zone of the chamber than they had done during the habituation phase (H). Throughout the test phase (T1-T3), the rats spent more time on contact with the tunnels than during the habituation phase (H).

*Differences between groups*. During the test phase, the Add rats spent more time in the transporter compared to their MovAdd counterparts. Rats from Mov and MovAdd groups interacted with the tunnels in the unchanged zone of the chamber more frequently than rats from Add group. There was also a difference in the frequency of moving between the chamber zones between the groups: MovAdd rats moved between the chamber zones more frequently than the rats from the Add group.

## 4. Discussion

The purpose of our experiment was to examine the impact of novelty on animal behavior where novelty involved increasing the complexity of the environment by adding elements and/or changing the properties of elements present in the environment. In this context, novelty is 'a change in stimulus conditions from previous experience' [53] (p. 189). It should be emphasized that the affordances in the form of tunnels that we used in our experiment respond to the needs of the species studied (rats), as they allow rats to engage in species-typical behaviors such as climbing on top of and hiding inside the tunnels. As in our previous studies [27], an account was taken of the ecological psychology dimension.

The animals' behavior differed between the groups, depending on the type of experimental manipulation used. In the Mov rats, no change in behavior was observed after the introduction of novelty. In this group, the manipulation involved changing the properties of the objects without increasing their number or arrangement between the habituation and test phase. On their first encounter with novelty, animals from this group could confront the change after a certain delay. The change (movability of the tunnels) was only discovered when the animals climbed the tunnel and made it move with their own body weight. It is likely that after seven days of habituation, the rats from the Mov group, due to the lack of spatial cues of the novelty, were slower to discover the change on the first test day as compared to the MovAdd group. The lack of distant cues resulted in the necessity of getting into direct proximity to the tunnel to reveal its new properties. They had to climb the tunnel and set it in motion. It may be assumed that before discovering the movability of the tunnel, the initial rats' expectations related to the tunnel in question would be the same as in the last habituation session [21]. In our study, these expectations were formed in the seven trial/days, long habituation phase, when the rats spent a 7-minute period in a stable test arena each day. They were subsequently exposed to unexpected changes (introduction of movable elements instead of stationary ones). If the change is not immediately conspicuous (new feature–movability–of otherwise unfamiliar tunnels), it may happen that it fails to elicit an immediate response since the ability to recognize it is hindered by previously formed expectations (cf. [54, 55]).

Rats from the Add and MovAdd groups spent significantly more time in the changed zone of the chamber after the introduction of novelty. The introduction of novelty changed

the animals' behavior in such a way that the rats spent much time near the objects, coming in contact with them, sniffing or climbing them; in this case, we could observe 'specific exploration', to use Berlyne's term [47]. The results we obtained may suggest, in line with what some researchers claim [56, 57], that novelty may have had a rewarding value. They also seem to confirm that under the conditions of low-stress experimental manipulation, animals are likely to investigate changes occurring in their environment ([18], p. 75) rather than exhibit a neophobic response [15]. The question remains as to why the addition of a new element in the MovAdd group resulted in a change of behavior, while no such change was observed in the Mov group described above. As demonstrated in one of our previous studies [27], rats engage in object-directed exploratory behavior where the complexity of the environment increases, i.e., when the number of tunnels goes up. The discrepancy between the expectations formed during the habituation phase and the changed environment encountered in the test phase results in a higher level of motivation to explore the new objects (cf. [21]). This allowed the MovAdd rats to detect the movability of the tunnels sooner than was the case in the Mov group, where the configuration of the tunnels did not change.

As regards the amount of time spent in the unchanged chamber and contact with the tunnels in that zone, no differences were observed in the Mov and Add groups between the habituation sessions and the test phase. However, the change was observed in the MovAdd group. Rats from that group increased the time spent in the unchanged zone and time on contact with tunnels in this zone after the introduction of novelty but decreased the time on the third test day. It seems that novelty combining move and addition of objects leads to a change of behaviour, whereas the move and addition provided separately do not have that effect. In the MovAdd group, the novelty the animals encountered had a higher degree of complexity but also a greater level of uncertainty than in the other groups. These features may have strengthened the rats' motivation to explore, resulting in less time being also spent in the familiar, unchanged zone of the chamber [25, 27, 28].

There is also a difference in the frequency of moving between the chamber zones between groups: rats from MovAdd group moved between the chamber zones more frequently than their Add counterparts. The differences between those two groups may be due to the fact that the movable element in the MovAdd group caused a certain degree of arousal and, therefore, elicited a need to reduce uncertainty by exploring different zones of the chamber.

A decrease in the amount of time spent in the transporter after the introduction of novelty was observed in all groups, which may suggest that the change did not invoke fear in the rats. However, the Add group rats spent more time in the transporter than their MovAdd counterparts in the test phase. It may be that the MovAdd rats, when faced with novelty characterized by a higher degree of complexity and uncertainty (increase in the number of tunnels *and* addition of a movable tunnel), devoted more time to exploring the tunnels. The Add rats (increase in the number of tunnels, no new and/or movable elements) quickly familiarized themselves with the new setting and did not devote their resources to exploring the environment.

In the MovAdd group, changes were observed in the amount of time spent in the changed zone of the chamber: rats from this group spent more time in that zone and on contact with the tunnels on each test day than during the habituation phase. On the third test day, however, they spent less time there than on the first test day. This could be explained by the fact that there were several things at play simultaneously: novelty in the form of a movable element motivated the animals to engage in exploration, but it could also constitute a source of uncertainty. Changes in a complex environment are often caused by factors unknown to the animal, and the associated uncertainty emerges when an organism encounters changes whose consequences it cannot predict. There is no certainty as to whether or not a particular event will occur again in the future or when. To a certain extent, uncertainty may increase the animal's

motivation to examine the changes as they occur [38, 58]. On the first test day, curiosity and the need to explore the environment prevailed over potential fear induced by the movable object, which may explain why the rats spent a lot of time in the changed zone of the chamber and on contact with the tunnels. Our procedure secures the low-stress or no-stress conditions, effectively reduces the neophobic responses. A series of previous studies have shown that the neophobic reactions are absent after a long familiarization to the experimental arena used in our protocol [50]. However, the rats' activity on the third test day decreased compared to the first test day. It appears that after some time, the movable element may have reduced the attractiveness of the environment which may be link to other factors than novelty of the objects, e.g., specific reaction to the movability. This phenomenon may be interpreted in terms of predation risk assessment [22], which in this case may have made interaction with the movable object more threatening than rewarding. Rodents and other small mammals can be prey for a variety of predators, including birds or other mammals. Thus, they must adapt to varying spatial predation risks [59]. Faced with a predatory threat, rodents, including rats, exhibit various behaviors such as flight, avoidance or freezing–species-specific defense response [60]. Confrontation with new objects or places is of key importance for the animal's survival–a novelty in the environment may provide new opportunities, but, equally, it may pose a threat [45].

The results obtained for the Mov group show no difference in the amount of time spent in the changed zone of the chamber and on contact with the tunnels between the habituation phase and the test phase. It seems, therefore, that the introduction of the movability of an element does not, in itself, result in a greater rewarding value of the changed tunnels, which renders this type of manipulation very different from changes involving spatial rearrangement or an increase in the complexity of the test environment. However, we would point out that the addition of movability and spatial rearrangement occurring at the same time (as in the MovAdd group) translated into changes in the behavioral characteristics observed. Our results demonstrate that environmental change involving the introduction of movability of the manipulated objects is not independent of, but rather interacts with, other parameters of the environment, such as its complexity and spatial arrangement. This conclusion is supported by the results of the analysis of the effect sizes, which show that the movability of the tunnels does not augment the positive effect of the increase in environmental complexity provided by the addition of tunnels but rather interacts with it in a complex multidirectional way.

Based on the results of our study, it can be concluded that the increase in environmental complexity contributes to animal welfare. Rats have the opportunity to carry out species-typical behaviors, which is manifested by an increase in exploratory behavior. However, it can be assumed that the introduction of movable elements can lead to increased environmental unpredictability and may contribute to elevated stress levels, at least at the initial stage of the familiarization with novel objects.

## 5. Conclusions

The results of our study show that the characteristics of the novelty introduced to the environment play a role in behavioral regulation in rats. The experimental manipulation we used involved different types of novelty: an increase in the test environment's complexity (addition of objects), a change in the properties of familiarized elements and a combination of these two scenarios.

The changes made to the test environment were 'affordance-inviting', to use a term coined by Withagen and colleagues [61]. The results obtained for the Add and MovAdd groups seem to corroborate this assumption. The results of our previous studies [27, 28] are also confirmed,

which showed that an increase in the complexity of the environment through the addition of tunnels triggers a more intensive exploratory activity in rats.

Our results indicate that the introduction of movability (as a new property of a familiar object) does not, as such, lead to a greater rewarding value being attributed to the changed tunnels. However, combining the introduction of movability with spatial rearrangement translates into changes in the behavioral characteristics observed. We would suggest that changing the environment by introducing the element of movability of the altered objects is not independent of, but rather interacts with, other parameters of the environment, such as its complexity and spatial arrangement.

## Supporting information

**S1 Data.**
(CSV)

## Author Contributions

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# Can the Hole–Board Test Predict a Rat's Exploratory Behavior in a Free-Exploration Test?

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**Simple Summary:** Since the introduction of the hole–board test, its validity and applicability have been repeatedly re-examined. The hole–board protocol remains one of the standard procedures applied in psychopharmacology and behavioral studies. Some authors advocate the use of the hole–board procedure in studies on various aspects of behavior regulation, such as exploration and anxiety, habituation to a novel environment, spatial learning and memory (working and reference memory), spatial pattern learning, and food search strategies. In this study, we focused on rats' activity in the hole–board test that we considered to be a type of exploratory activity. Based on our results and our previous studies of rats' exploratory behavior in the free-exploration box, we suggest that the hole–board apparatus might not be the best tool for measuring exploratory behavior in laboratory rodents.

**Abstract:** This study focuses on the rat activity in a hole–board setting that we considered a type of exploratory behavior. The general hypothesis is based on the claim that a motivational mechanism is central to both the response to novelty in a highly familiarized environment and the activity in the hole–board apparatus. Our sample consisted of 80 experimentally naive Lister Hooded rats. All rats were tested in the hole–board apparatus. Twenty individuals with the highest hole-board scores and twenty subjects with the lowest hole–board scores subsequently underwent an established free-exploration test. In our study, the scores obtained in the hole–board test had little predictive value for the rats' activity in the free-exploration test. Based on our previous experience in studying exploratory behavior in the free-exploration test and the data presented in this paper, we suggest that the hole–board test is not an appropriate tool for measuring exploratory behavior in laboratory rodents.

**Keywords:** hole–board; free-exploration test; exploratory behavior; rat



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## 1. Introduction

The hole–board test is a behavioral test that has been used to assess different aspects of cognitive abilities and emotions in small mammals. It originated from an open field test, which was designed to evaluate exploratory behavior and anxiety [1]. The hole–board apparatus has small cylindrical holes at the bottom of the experimental arena that allows experimenters to conduct more complex behavioral observations. A head-dipping in the holes is considered a main feature of the behavior in this experimental arena. However, since the introduction of the hole–board test [2], its validity and applicability have been repeatedly re-examined. Head-dipping in the hole was considered by File and Wardill [3] to be a valid measure of exploratory activity. The discussion continued, and R. Hughes [4] (p. 449) stated: "There is also the possibility that head-dipping could involve attempts to find an escape route rather than reflect a genuine interest in objects underneath the holes". This view was substantiated by an experimental study on Lister-Hooded rats [5] (p. 442) in which the authors concluded: "Rather than being a measure of neophilia, these results

support the hypothesis that head-dipping represents an escape response, which declines as the subject becomes less fearful". Despite this controversy, the hole–board protocol remains one of the standard procedures applied in psychopharmacology and behavioral studies. Some authors [6] advocate the use of the hole–board procedure in studies on various aspects of behavior regulation (such as exploration and anxiety, habituation to a novel environment, spatial learning and memory (working and reference memory), spatial pattern learning, and food search strategies) [7,8]. More recently, the hole–board procedure has also been used to assess behavioral characteristics that are supposed to reflect an animal model of Autism spectrum disorder (ASD) [9].

Neophillia (the tendency to approach unfamiliar objects or environments—[10,11]) in animal behavior has long been a subject of scientific discussion. We share the view expressed in earlier papers that any behavior in a novel environment would be regulated by both neophilia and neophobia [5,6,12,13]. Therefore, rather than being at the two extremes of a continuum, neophilia and neophobia should be thought of as two independent mechanisms that can come into play simultaneously. Over the past three decades, research on curiosity and exploration in animals has established some basic methodological guidelines for further studies. The experimental stimulus of novelty should be controlled by ensuring a sufficiently long habituation period. Moreover, the test environment should provide the subjects with a comfortable low-stress setting, which does not trigger neophobic or defense responses. A validated protocol of this methodological approach has already been established [14], and a detailed description of the free-exploration test was published [15], enabling readers to fully replicate all of the procedural details. Since the ecological validity of the protocol for neophillia and the exploratory responses has been clearly demonstrated, it suggests that the free-exploration test may serve as a tool for evaluating other methods of behavioral assessments in terms of their validity for measuring exploratory behavior. Since the hole–board procedure is often used as a tool for measuring exploratory behavior in rodents, our study sought to validate the two protocols in question, namely the hole–board protocol and the free-exploration test (low-stress), as previously described [15].

To date, there are many variants of the hole–board devices. We chose the most standard and widely used apparatus. While the hole–board procedure is used for numerous purposes (such as anxiety or general activity assessment or behavioral profiling), we have focused on the exploratory aspect of the behavior in the rats' activity on the hole–board. The general hypothesis was based on the claim that there is a motivational mechanism central to both the response to novelty in a highly familiarized environment and the activity in the hole–board apparatus. If it is true, there should be a strong positive correlation between both kinds of activity, namely the response to novelty in the free-exploration box and the activity in the hole–board apparatus.

## 2. Materials and Methods

### *2.1. Animals*

The sample consisted of 80 experimentally naive Lister Hooded rats. The rats were bred and kept in the vivarium of the Institute of Psychology, Polish Academy of Sciences, Warsaw, Poland. The rats were approximately 90 days old and weighed approximately 350 g at the start of the experiment.

The rats were housed in groups of 3–4 in Tecniplast Eurostandard Type IV cages (610 mm × 435 mm × 215 mm) with dust-free softwood granules (Tierwohl Super, Rosenberg, Germany) as bedding and with ad libitum access to water and standard laboratory fodder (Labofeed H, WP Morawski, Kcynia, Poland). The day/night cycle was set at 12/12 h (with the lights on at 8 a.m.) and the temperature was maintained at a constant 21–23 °C. The cages and pens were cleaned once a week, on the same day and at a fixed time, in the late afternoon (5 p.m.). However, in order to ensure that the experimental procedure was not disturbed, the cages in which the test animals were kept were cleaned just before the onset of the experiment and again after the experiment was finished. All of the rats kept in our laboratory were housed, bred and taken care of in accordance with the Regulation

of the Polish Minister for Agriculture and Rural Development of 14 December 2016 on laboratory animal care. The experimental procedures were approved by the First Local Ethics Committee on Animal Experimentation in Warsaw, Poland (No. 1116/2020).

The sample size was estimated using a commonly used formula for calculating sample size for repeated measures [16]:

$$N = 1 + C(s/d)^2$$

where:

s—standard deviation of the population means

d—size of difference in means (the effect)

C—constant dependent on the value of α (significant level) and 1-ß (power)

For the purpose of our study, we employed the following parameters: $\alpha = 0.05$; $\beta = 0.20$ ($C = 10.51$).

Group size calculations were carried out on the basis of a previous study [14] in which the average time spent on exploring changed objects was $M = 96.51$, with standard deviation $s = 36.63$, and on the assumption that the detectable difference between the variables should be $d = 41$.

Therefore, the total sample size for the free-exploration test was estimated at 10.

### *2.2. Procedure*

The experiment was conducted in two phases. In Phase I, a hole–board test was carried out. Then, the selected animals were subjected to the exploration test in Phase II.

#### 2.2.1. Phase I—The Hole–board Test

The aim of the test was to measure the rats' activity (i.e., head-dipping behavior) in the experimental area and select individuals with the lowest and the highest levels of analyzed behavior. The scores were assigned based on the number of instances of head-dipping in the holes during a 5-min session. Twenty subjects (10 males and 10 females) with the highest scores and twenty individuals (10 males and 10 females) with the lowest scores on the hole–board test were admitted to Phase II.

We used a square hole–board arena measuring 600 × 600 × 450 mm (Hole–board for rats, manufactured by MazeEngineers, Skokie, IL, USA) (Figure 1). The transparent Plexiglas walls were covered to prevent the animals from being distracted by visual stimuli. There were 16 round-shaped holes in the bottom of the arena, each 50 mm in diameter, distributed evenly at equal distances from each other. The experimental arena was illuminated by fluorescent ceiling lamps, set at approximately 75–100 lx. The video camera used for recording the rats' behavior was placed 1.2 m above the measurement apparatus.

After being taken out of the housing cage, each rat was immediately placed in a transporter (a small cylindrical cage 60 mm in diameter with doors 120 mm high and 100 mm wide) and moved to another room where the experiment took place. The transporter with the animal was placed in the corner of the experimental arena in such a way that the rats could leave the transporter through the exit into the interior of the arena. After the transporter exit was opened, each animal was left in the arena for five minutes. Each rat was free to explore the experimental arena and leave and enter the transporter without impediment. After completing the session, the animal was removed from the experimental arena and returned to its home cage. The hole–board test was carried out once. The testing arena was cleaned after each rat using Virkon S (Bayer, Leverkusen, Germany).

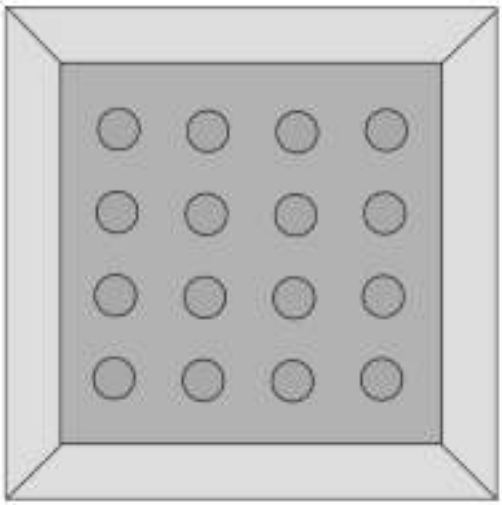

**Figure 1.** Hole–board apparatus (view from above).

2.2.2. Phase II—The Free-Exploration Test

One day after the hole–board test, the selected (high-activity and low-activity) rats participated in the free-exploration test. The high-activity group included 20 individuals (10 males and 10 females) that had the highest scores in the hole–board test whereas the low-activity group included another 20 individuals (10 males and 10 females) that had the lowest scores in the test.

The aim of the free-exploration test was to measure the differences between the process of exploring the new environment, the rate of habituation to it, and the response to the introduction of harmless novelty into the familiar context. The apparatus and the measurement methods were similar to those used in our previous studies (e.g., [14,15,17–19]).

The experimental chamber was a box with dimensions of 800 mm × 600 mm × 800 mm. The space inside the chamber was divided into three zones (A–C), separated by two walls diametrically perpendicular to its longer side (Figure 2). The front part of the chamber was a transparent wall that could be elevated in order to gain full access to the testing arena. The wooden partition walls between the zones had triangular passages (120 mm × 140 mm) at the bottom, which allowed animals to move freely between parts of the chamber. The entire chamber was coated with a layer of removable varnish. Wooden tunnels (200 mm × 120 mm × 80 mm) covered with washable paint were positioned in zones B and C. Unlike the most commonly used two-dimensional experimental settings, these tunnels provided a complex three-dimensional environment. The middle zone (A) was empty. There was a hole in the back wall of the chamber that functioned as an entrance for animals moving from the animal transporter to the chamber. The transporter functioned as a starter box (60 mm in diameter with doors 120 mm high and 100 mm wide).

At the beginning of each trial, the transporter with the animal inside was placed at the entrance to Zone A. Then, the rat was left unhindered in the starting box for 15 s, after which the entrance door was opened. The animal was allowed to stay in the starting box or leave it to examine the chamber. The first seven trials were habituation trials, during which the apparatus was adjusted in exactly the same way (Figure 3). The implementation of the novelty took place on trial 8. The novelty took the form of adding additional tunnels, as it is shown in the right panel of Figure 3. Three consecutive tests trials were carried out with the chamber in this new configuration.

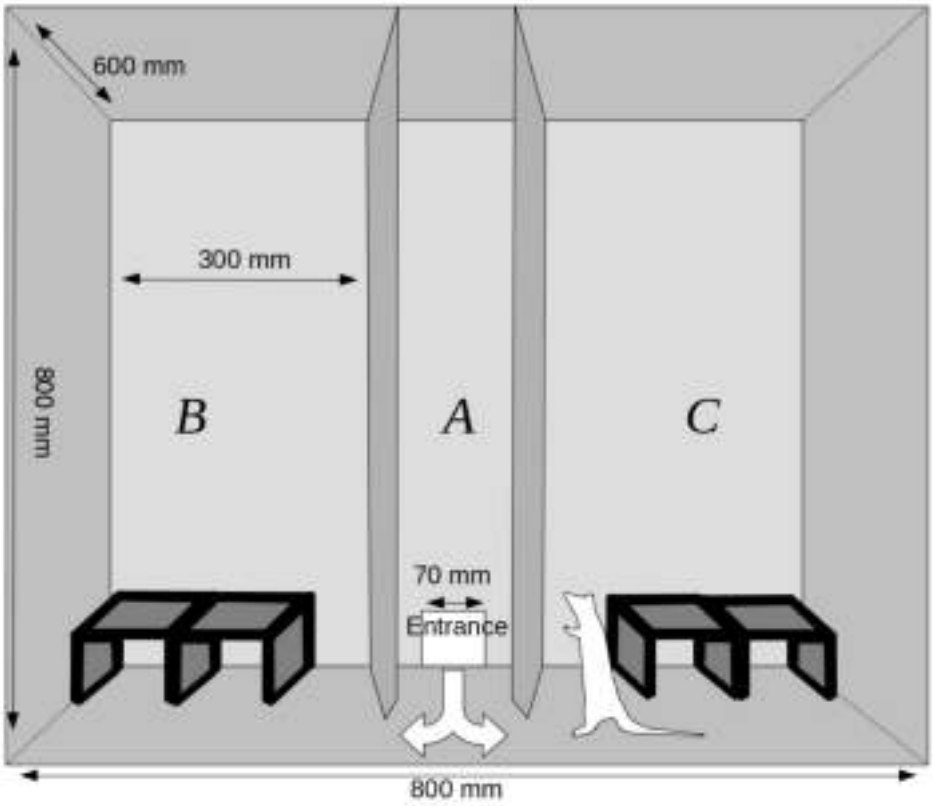


**Figure 2.** The experimental chamber of the free-exploration test—frontal view through the transparent front wall.

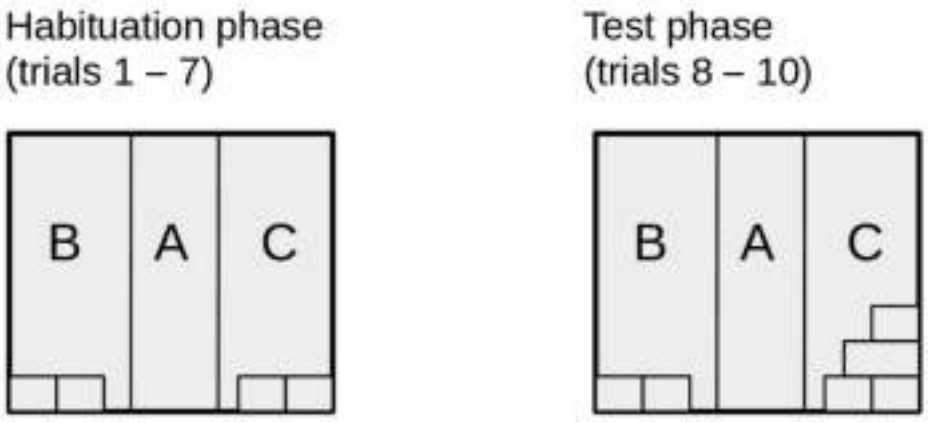


**Figure 3.** The arrangement of objects in the experimental chamber in each experimental setting. Habituation phase—two tunnels placed next to each other in sections B and C; Test phase—two tunnels placed next to each other in section B and two tunnels placed next to each other and an additional two tunnels placed at the top in section C.

Each trial lasted 7 min and was carried out for each animal once a day. Each experimental session was followed by a cleaning of the experimental arena, the tunnels, and the transporter using Virkon S (Bayer) in order to eliminate odors left by the previous animal.

A video camera was placed at a distance of approximately 1.5 m from the transparent front wall of the experimental chamber. The camera was set up in night-time shooting mode to ensure the possibility of filming in the dark.

To prevent the effects of lateralization or visual/auditory cues, novel objects were introduced in the left zone (as described above) for half of the examined subjects and in the right zone for the other half (mirror image of Figure 3).

### *2.3. Data Processing and Statistical Analyses*

To code the behaviors on the basis of the recorded material, we used BORIS software (http://www.boris.unito.it), which made it possible to define selected behaviors and to assess their duration and frequency. We scored the behaviors the animals engaged in during the entire experimental trial. Consequently, we were able to assign specific scores to the times of separate bouts of behaviors, their frequency, and the total time an animal spent engaging in a given behavior. The following variables were measured: (1) time spent in the transporter (excluding the latency required to leave the transporter); (2) time spent in the central zone; (3) time spent in the unchanged zone of the chamber; (4) time spent in the changed zone of the chamber; (5) frequency of moving between the zones (left/right/transporter) of the chamber; (6) time spent in contact with the tunnels in the unchanged zone of the chamber; (7) frequency of contact with the tunnels in the unchanged zone of the chamber; (8) time spent in contact with the tunnels in the changed zone of the chamber; and (9) frequency of contact with the tunnels in the changed zone of the chamber. The time spent in the experimental chamber was measured in seconds.

To enhance the legibility of the results and tables, the habituation phase has been indicated as H (the mean score from habituation trials 5 through 7 that presents the outcome of the process of habituation to the experimental environment and served as a reference value for further analyses), while the test trials have been indicated as T1, T2, and T3, respectively. Novelty (i.e., the addition of tunnels in zone C) was introduced in the first test trial (T1). Groups selected on the basis of the activity in the hole–board test were named as follows: the high-activity group was known as HB_H and the low-activity group was known as HB_L.

The data were analyzed using a general linear model procedure (GLM), with repeated measurements (H, T1, T2, T3) as within-subject factors, as well as sex and hole–board group assignment as between-subject factors. This was followed by post-hoc *t*-tests with Bonferroni correction for multiple comparisons. Differences were considered significant for $p \leq 0.05$.

## 3. Results

The first-step analysis was designed to extract the individuals of the high and low levels of activity (i.e., head-dipping) in the Hole–board apparatus. Table 1 shows the descriptive statistics of this measurement. Individuals falling below the 25th percentile and above the 75th percentile were qualified for further tests.

**Table 1.** Descriptive statistics of the rats' head-dipping activity in the hole–board apparatus (Phase I).

| Descriptive Statistics | Female | Male |
|---|---|---|
| Valid cases | 40 | 40 |
| Missing cases | 0 | 0 |
| Mean | 63.125 | 65.850 |
| Std. Deviation | 12.081 | 21.832 |
| Minimum | 36.000 | 22.000 |
| Maximum | 82.000 | 117.000 |
| 25th percentile | 55.750 | 50.500 |
| 50th percentile | 66.000 | 65.000 |
| 75th percentile | 72.000 | 80.250 |

The complete set of descriptive statistics of all the free-exploration test variables analyzed is shown in Table 2.

**Table 2.** Descriptive statistics of the variables analyzed in the free-exploration test (Phase II).

| Trial | Female | | | | Male | | | |
|---|---|---|---|---|---|---|---|---|
| | HB_H | | HB_L | | HB_H | | HB_L | |
| | Mean | Stdv | Mean | Stdv | Mean | Stdv | Mean | Stdv |
| Time spent in the transporter (seconds) | | | | | | | | |
| H | 53.489 | 16.865 | 78.091 | 21.987 | 57.950 | 18.938 | 71.255 | 30.743 |
| T1 | 25.497 | 14.998 | 33.446 | 24.695 | 21.100 | 10.088 | 28.471 | 10.855 |
| T2 | 27.500 | 11.119 | 39.975 | 25.770 | 38.097 | 13.819 | 38.350 | 25.722 |
| T3 | 31.250 | 9.284 | 19.147 | 8.359 | 62.600 | 20.514 | 40.725 | 17.311 |
| Time spent in the central zone of the chamber (seconds) | | | | | | | | |
| H | 87.382 | 17.671 | 129.922 | 18.244 | 103.405 | 28.115 | 131.752 | 31.083 |
| T1 | 42.113 | 16.224 | 86.806 | 26.956 | 65.422 | 28.689 | 88.218 | 30.795 |
| T2 | 44.400 | 22.570 | 92.755 | 31.475 | 62.176 | 17.438 | 90.301 | 35.716 |
| T3 | 86.138 | 40.483 | 86.452 | 32.073 | 83.218 | 26.809 | 100.955 | 30.661 |
| Time spent in the unchanged zone of the chamber (seconds) | | | | | | | | |
| H | 118.484 | 15.684 | 80.812 | 18.546 | 142.643 | 28.925 | 128.811 | 36.466 |
| T1 | 69.950 | 29.519 | 52.200 | 22.475 | 77.350 | 20.072 | 77.278 | 36.888 |
| T2 | 79.050 | 26.858 | 97.819 | 36.724 | 93.001 | 31.368 | 63.225 | 35.026 |
| T3 | 80.276 | 27.460 | 107.424 | 38.002 | 78.147 | 29.488 | 81.350 | 32.115 |
| Time spent in the changed zone of the chamber (seconds) | | | | | | | | |
| H | 158.247 | 28.266 | 127.658 | 21.996 | 113.634 | 22.628 | 85.126 | 17.594 |
| T1 | 279.091 | 43.538 | 244.774 | 43.715 | 254.278 | 37.004 | 223.808 | 33.103 |
| T2 | 268.749 | 26.178 | 186.350 | 33.690 | 223.852 | 40.256 | 226.049 | 63.430 |
| T3 | 219.426 | 46.717 | 203.725 | 44.906 | 193.411 | 28.945 | 194.200 | 48.507 |
| Frequency of moving between the zones (left/right/transporter) of the chamber | | | | | | | | |
| H | 16.600 | 4.537 | 18.200 | 2.654 | 19.100 | 2.648 | 18.500 | 4.238 |
| T1 | 13.500 | 5.462 | 17.000 | 3.367 | 15.200 | 3.011 | 15.700 | 2.869 |
| T2 | 11.800 | 4.341 | 16.200 | 3.490 | 17.000 | 3.266 | 15.400 | 2.836 |
| T3 | 15.400 | 3.596 | 15.400 | 2.989 | 17.000 | 3.528 | 15.500 | 1.780 |
| Time spent in contact with the tunnels in the unchanged zone of the chamber (seconds) | | | | | | | | |
| H | 81.598 | 12.940 | 47.508 | 14.835 | 98.234 | 23.204 | 80.869 | 39.429 |
| T1 | 48.350 | 23.602 | 30.000 | 14.163 | 51.704 | 14.573 | 48.825 | 29.438 |
| T2 | 61.747 | 22.725 | 74.880 | 37.181 | 67.179 | 25.447 | 39.950 | 32.483 |
| T3 | 63.954 | 25.023 | 80.030 | 32.701 | 56.068 | 20.425 | 55.650 | 30.935 |
| Frequency of contact with the tunnels in the unchanged zone of the chamber | | | | | | | | |
| H | 6.533 | 1.492 | 5.133 | 0.706 | 8.733 | 2.372 | 7.367 | 1.222 |
| T1 | 4.500 | 1.958 | 4.700 | 1.636 | 6.300 | 1.567 | 5.400 | 1.776 |
| T2 | 4.800 | 1.398 | 4.900 | 1.370 | 6.800 | 1.989 | 4.600 | 1.174 |
| T3 | 4.700 | 1.337 | 5.200 | 0.632 | 6.100 | 2.079 | 5.400 | 1.174 |
| Time spent in contact with the tunnels in the changed zone of the chamber (seconds) | | | | | | | | |
| H | 111.982 | 19.581 | 76.694 | 14.877 | 75.809 | 18.746 | 51.297 | 17.797 |
| T1 | 248.350 | 43.400 | 200.213 | 40.112 | 220.723 | 40.276 | 175.604 | 35.720 |
| T2 | 243.399 | 30.898 | 152.952 | 32.536 | 192.424 | 37.455 | 182.876 | 56.932 |
| T3 | 195.926 | 46.201 | 166.971 | 44.976 | 164.299 | 23.887 | 157.450 | 48.469 |
| Frequency of contact with the tunnels in the changed zone of the chamber | | | | | | | | |
| H | 7.700 | 1.836 | 7.500 | 1.672 | 7.100 | 1.277 | 6.333 | 1.812 |
| T1 | 8.400 | 2.716 | 10.100 | 1.853 | 8.200 | 2.150 | 8.900 | 2.424 |
| T2 | 6.900 | 1.370 | 7.600 | 0.966 | 9.600 | 2.171 | 8.900 | 2.726 |
| T3 | 8.000 | 2.261 | 8.500 | 2.550 | 9.000 | 2.867 | 8.800 | 3.458 |

HB_H—high-activity group; HB_L—low-activity group; Stdv—standard deviation; H—habituation trials; T1—first test trial; T2—second test trial; T3—third test trial.

### *3.1. Time Spent in the Transporter*

The analysis showed significant trial by sex interaction (Wilks' Lambda; F(3,105) = 7.684; $p \leq 0.001$; eta$^2$ = 0.180), trial by group interaction (F(3,105) = 8.875; $p \leq 0.001$; eta$^2$ = 0.202) and a main effect of the trial (Wilks' Lambda; F(3,105) = 41.646; $p \leq 0.001$; eta$^2$ = 0.543).

A post-hoc analysis of sex interaction showed a significant decrease in the time spent in the transporter in T1 compared to the habituation phase in females ($p \leq 0.001$, $M_H = 65.79$, $SD_H = 22.87$, $M_{T1} = 29.47$, $SD_{T1} = 20.30$, Cohen's d = 1.120) and in males ($p \leq 0.001$, $M_H = 64.60$, $SD_H = 25.77$, $M_{T1} = 24.79$, $SD_{T1} = 10.88'$ Cohen's d = 1.275). In females during the next trials, the amount of time spent in the transporter remained at the same low level. In males, there was an increase in time spent in the transporter between T1 and T3 ($p \leq 0.001$, $M_{T3} = 51.66$, $SD_{T3} = 21.61$, Cohen's d = 0.861).

A post-hoc analysis of group interaction showed a significant decrease in the time spent in the transporter in T1 compared to the habituation phase in the HB_high group ($p \leq 0.001$, $M_H = 55.72$, $SD_H = 17.60$, $M_{T1} = 23.30$, $SD_{T1} = 12.64$, Cohen's d = 0.998) and in the HB_low group ($p \leq 0.001$, $M_H = 74.67$, $SD_H = 26.36$, $M_{T1} = 30.96$, $SD_{T1} = 17.77$, Cohen's d = 1.400).

### *3.2. Time Spent in the Central Zone of the Chamber*

The analysis showed a significant main effect of the trial (Wilks' Lambda; F(3,108) = 20.910; $p \leq 0.001$; $eta^2 = 0.367$), and a main effect of the group (Wilks' Lambda; F(1,36) = 37.157; $p \leq 0.001$; $eta^2 = 0.508$).

A post-hoc analysis showed a significant decrease in time spent in the central zone in T1 compared to that in the habituation phase ($p \leq 0.001$, Cohen's d = 1.102). Subjects from the HB_high group spent less time in that zone than did subjects from the HB_low group ($p \leq 0.001$, Cohen's d = 0.964).

### *3.3. Time Spent in the Unchanged Zone of the Chamber*

The analysis showed significant sex by group interaction (Wilks' Lambda; F(3,108) = 4.891; $p = 0.003$; $eta^2 = 0.120$), trial by group interaction (F(3,108) = 4.60; $p = 0.004$; $eta^2 = 0.114$), trial by sex interaction (F(3,108) = 9.183; $p \leq 0.001$; $eta^2 = 0.203$), and a main effect of the trial (Wilks' Lambda; F(3,108) = 27.449; $p \leq 0.001$; $eta^2 = 0.433$).

A post-hoc analysis showed a significant decrease in the time spent in the unchanged zone in trial T1 compared to that spent during the habituation in females from the HB_high group ($p = 0.003$, $M_H = 118.48$, $SD_H = 15.68$, $M_{T1} = 69.95$, $SD_{T1} = 29.52$, Cohen's d = 0.696), in males from the HB_high group ($p \leq 0.001$, $M_H = 142.64$, $SD_H = 28.92$, $M_{T1} = 77.35$, $SD_{T1} = 20.07$, Cohen's d = 0.936), and in males from the HB_low group ($p \leq 0.001$, $M_H = 128.81$, $SD_H = 36.47$, $M_{T1} = 77.28$, $SD_{T1} = 36.89$, Cohen's d = 0.739), but not in females from the HB_low group. However, there was an increase in time spent in the unchanged zone between T1 and T2 ($p = 0.008$, $M_{T1} = 52.20$, $SD_{T1} = 22.47$, $M_{T2} = 97.82$, $SD_{T2} = 36.72$, Cohen's d = 0.654) and between T1 and T3 in that group ($p \leq 0.001$, $M_{T3} = 107.42$, $SD_{T3} = 38.00$, Cohen's d = 0.792) in females from the HB_low group.

### *3.4. Time Spent in the Changed Zone of the Chamber*

Mauchly's test indicated that the assumption of sphericity had been violated ($\chi 2(5) = 12.43$, $p = 0.029$), so the degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity ($\varepsilon = 0.85$). The analysis showed significant trial by sex by group interaction (F(2.547,108) = 2.893; $p = 0.048$; $eta^2 = 0.074$) and a main effect of the trial (F(2.547,108) = 95.496; $p \leq 0.001$; $eta^2 = 0.726$).

A post-hoc analysis showed a significant increase in the time spent in the changed zone in trial T1 compared to the habituation in females from the HB_high group ($p \leq 0.001$, $M_H = 158.25$, $SD_H = 28.27$, $M_{T1} = 279.09$, $SD_{T1} = 43.54$, Cohen's d = 1.176) and from the HB_low group ($p \leq 0.001$, $M_H = 127.66$, $SD_H = 22.00$, $M_{T1} = 244.77$, $SD_{T1} = 43.71$, Cohen's d = 1.139), in males from the HB_high group ($p \leq 0.001$, $M_H = 113.63$, $SD_H = 22.63$, $M_{T1} = 254.28$, $SD_{T1} = 37.00$, Cohen's d = 1.386), and in males from the HB_low group ($p \leq 0.001$, $M_H = 85.13$, $SD_H = 17.59$, $M_{T1} = 223.81$, $SD_{T1} = 33.10$, Cohen's d = 1.349). There was a decrease in time spent in the changed zone between T1 and T3 in females from the HB_high group ($p = 0.045$, $M_{T3} = 219.43$, $SD_{T3} = 46.72$, Cohen's d = 0.580) and in males from the HB_high group ($p = 0.035$, $M_{T3} = 193.41$, $SD_{T3} = 28.94$, Cohen's d = 0.592).

There were also differences between females from the HB_low and the HB_high group in T2 ($p \leq 0.001$, $M_{HB\_low} = 186.35$, $SD_{HB\_low} = 33.69$, $M_{HB\_high} = 268.75$, $SD_{HB\_high} = 26.18$, Cohen's d = 0.765).

### *3.5. Frequency of Moving between the Zones (Left/Right/Transporter) of the Chamber*

The analysis showed a significant main effect of the trial (Wilks' Lambda; F(3,108) = 8.930; $p \leq 0.001$; $eta^2 = 0.199$).

A post-hoc analysis showed a significant decrease in frequency of moving between the zones in trial T1 as compared to that during the habituation phase ($p \leq 0.001$, Cohen's d = 0.670).

The analysis of between subject effects revealed an interaction effect of sex and group (F(1,36) = 4.146; $p = 0.049$; $eta^2 = 0.103$). However, post-hoc comparisons did not reveal any specific differences between the groups.

### *3.6. Time Spent in Contact with the Tunnels in the Unchanged Zone of the Chamber*

The analysis showed significant trial by sex by group interaction (Wilks' Lambda; F(3,108) = 4.193; $p = 0.008$; $eta^2 = 0.104$), trial by group interaction (F(3,108) = 4.210; $p = 0.007$; $eta^2 = 0.105$), trial by sex interaction (F(3,108) = 9.013; $p \leq 0.001$; $eta^2 = 0.200$), and a main effect of the trial (Wilks' Lambda; F(3,108) = 15.663; $p \leq 0.001$; $eta^2 = 0.303$).

A post-hoc analysis showed a significant decrease the time spent in contact with the tunnels in the unchanged zone in trial T1 compared to the habituation trials in males from the HB_high group ($p \leq 0.001$, $M_H = 98.23$, $SD_H = 23.20$, $M_{T1} = 51.70$, $SD_{T1} = 14.57$, Cohen's d = 0.775), but not in males from the HB_low group or in females from either experimental group. An increase in females from the HB-low group in trial T2 ($p \leq 0.001$, $M_{T1} = 30.00$, $SD_{T1} = 14.16$, $M_{T2} = 74.88$, $SD_{T2} = 37.18$, Cohen's d = 0.428) and T3 ($p \leq 0.001$, $M_{T3} = 80.03$, $SD_{T3} = 32.70$, Cohen's d = 0.833) compared to trial T1 was also shown.

### *3.7. Frequency of Contact with the Tunnels in the Unchanged Zone of the Chamber*

The analysis showed a significant main effect of the trial (Wilks' Lambda; F(3,108) = 15.463; $p \leq 0.001$; $eta^2 = 0.300$), a main effect of sex (F(1,36) = 14.877; $p \leq 0.001$; $eta^2 = 0.292$) and a main effect of the group (Wilks' Lambda; F(1,36) = 4.724; $p = 0.036$; $eta^2 = 0.116$).

A post-hoc analysis showed a significant decrease in frequency of contact with the tunnels in the unchanged zone in trial T1 compared to that in the habituation phase ($p \leq 0.001$, Cohen's d = 0.908). Additionally, males more frequently interacted with the tunnels in the unchanged zone than did females ($p \leq 0.001$, Cohen's d = 0.610), and subjects from the HB_high group more frequently interacted with the tunnels than did subjects from the HB_low group ($p = 0.036$, Cohen's d = 0.344).

### *3.8. Time Spent in Contact with the Tunnels in the Changed Zone of the Chamber*

Mauchly's test indicated that the assumption of sphericity had been violated ($\chi 2(5) = 12.085$, $p = 0.034$), so the degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity ($\varepsilon = 0.85$). The analysis showed a significant main effect of the trial (F(2.554,108) = 111.948; $p \leq 0.001$; $eta^2 = 0.757$).

A post-hoc analysis showed a significant increase in time spent in contact with tunnels in the change zone between H and T1 trials ($p \leq 0.001$, Cohen's d = 2.664) and between T1 and T3 trials ($p \leq 0.001$, Cohen's d = 0.807).

The analysis of between subject effects revealed an interaction effect of sex and group (F(1,36) = 5.164; $p = 0.029$; $eta^2 = 0.125$). On the basis of the post-hoc comparisons, it was found that females from the HB_high group spent more time on interaction with the tunnels than did females from the HB_high group ($p \leq 0.001$, Cohen's d = 0.882), and males from the HB_high ($p = 0.002$, Cohen's d = 0.637) and the HB_low ($p \leq 0.001$, Cohen's d = 1.011) groups.

### *3.9. Frequency of Contact with the Tunnels in the Changed Zone of the Chamber*

The analysis showed significant trial by sex interaction (Wilks' Lambda; F(3,108) = 5.386; $p$ = 0.002; eta$^2$ = 0.130), and a main effect of the trial (Wilks' Lambda; F(3,108) = 6.838; $p \leq 0.001$; eta$^2$ = 0.160).

A post-hoc analysis showed a significant decrease in frequency of contact with the tunnels in the changed zone in females between the T1 and T2 trials ($p$ = 0.022, $M_{T1}$ = 9.25, $SD_{T1}$ = 2.42, $M_{T2}$ = 7.25, $SD_{T2}$ = 1.21, Cohen's d = 0.547).

### *3.10. Effect Size Analysis*

To allow the reader to compare the powers of the effects found in our study, Table 3 shows effect size estimations.

**Table 3.** The ranking list of statistically significant effects based on the partial Eta$^2$ values.

| Variable | Effect of: | Eta$^2$ |
|---|---|---|
| Time spent in contact with the tunnels in the changed zone of the chamber | trial | 0.757 |
| Time spent in the changed zone of the chamber | trial | 0.726 |
| Time spent in the unchanged zone of the chamber | trial | 0.433 |
| Time spent in the central zone of the chamber | trial | 0.367 |
| Time spent in contact with the tunnels in the unchanged zone of the chamber | trial | 0.303 |
| Frequency of contact with the tunnels in the unchanged zone of the chamber | trial | 0.300 |
| Frequency of contact with the tunnels in the unchanged zone of the chamber | sex | 0.292 |
| Time spent in the unchanged zone of the chamber | trial by sex | 0.203 |
| Time spent in contact with the tunnels in the unchanged zone of the chamber | trial by sex | 0.200 |
| Frequency of moving between the zones (left/right/transporter) of the chamber | trial | 0.199 |
| Time spent in the transporter | trial by sex | 0.180 |
| Frequency of contact with the tunnels in the changed zone of the chamber | trial by sex | 0.130 |
| Time spent in contact with the tunnels in the changed zone of the chamber | sex by group | 0.125 |
| Time spent in the unchanged zone of the chamber | trial by sex by group | 0.120 |
| Frequency of contact with the tunnels in the unchanged zone of the chamber | group | 0.116 |
| Time spent in the unchanged zone of the chamber | trial by group | 0.114 |
| Time spent in contact with the tunnels in the unchanged zone of the chamber | trial by group | 0.105 |
| Time spent on contact with the tunnels in the unchanged zone of the chamber | sex by group | 0.104 |
| Time spent in the changed zone of the chamber | trial by sex by group | 0.074 |

A descriptive summary of the results is shown in Table 4.

**Table 4.** Descriptive non-statistical summary of the results.

| Effect Code | Description of Effect |
|---|---|
| | Time spent in the transporter |
| Trial × Sex | The general pattern of the response was similar in both females and males. All subjects spent less time in the transporter in T1. Males, however, spent more time staying in the transporter in T3. |
| Trial × Group | The general pattern of the response was similar in both HB groups. All subjects spent more time in the transporter in sessions T1 than T3. However, in subjects from the HB-low groups, this tendency was more pronounced. |
| | Time spent in the central zone of the chamber |
| Trial | All rats spent less time in the central zone in trial T1 compared to that spent in the habituation phase. |
| Group | Subjects from the HB_high group spent less time in that zone than did subjects from the HB_low group across all experimental trials. |
| | Time spent in the unchanged zone of the chamber |
| Trial × Sex × Group | HB_high females and all males spent less time in the unchanged zone of the chamber in trial T1. However, no such effect was observed in females from the HB_low group. On the contrary—there was an increase in the amount of time spent in the unchanged zone between T2 and T3. |

**Table 4.** *Cont.*

| Effect Code | Description of Effect |
| --- | --- |
| | Time spent in the changed zone of the chamber |
| Trial × Sex × Group | For all rats, there was an increase in the duration of staying in the changed zone of the chamber in trial T1. However, both females and males from the HB_high groups spent less time in the changed zone in trial T3 as compared to trial T1. The duration of staying in the chamber's changed zone in HB_high individuals was generally longer than in HB_low individuals, which was most clearly manifested within the female subsample in trial T2. |
| | Frequency of moving between the zones (left/right/transporter) of the chamber |
| Trial | For all rats, there was a decrease in the frequency of moving between the zones in trial T1. |
| | Time spent in contact with the tunnels in the unchanged zone of the chamber |
| Trial × Sex × Group | HB_high male rats spent less time in contact with tunnels in the unchanged zone, but HB_low male rats and all females did not show this pattern. Females from the HB_low group spent more time in contact with the tunnels in this zone in trials T2 and T3 as compared to trial T1. |
| | Frequency of contact with the tunnels in the unchanged zone of the chamber |
| Trial | All rats interacted with the tunnels in the unchanged zone in trial T1 less frequently than they did in the habituation trials. |
| Sex | Males interacted with the tunnels in the unchanged zone more frequently than females across all experimental trials. |
| Group | HB_high subjects interacted with the tunnels in the unchanged zone more frequently than their HB_low counterparts across all experimental trials. |
| | Time spent in contact with the tunnels in the changed zone of the chamber |
| Trial | All individuals spent more time interacting with the tunnels in the changed zone in trial T1. |
| Sex × Group | HB_high females spent more time interacting with the tunnels in the changed zone than all other counterparts. |
| | Frequency of contact with the tunnels in the changed zone of the chamber |
| Trial × Sex | All subjects responded to tunnel modification with an increase in the frequency of contact with the tunnels in trial T1. However, there was a decrease in the time spent by females on this activity in trial T2. |

## 4. Discussion

In this study, we focused on rats' activity on the hole–board, which we considered to be a type of exploratory activity. This approach is based on a long-standing theoretical tradition [20], which is still being developed nowadays [21]. The general hypothesis was based on the claim that motivational mechanisms are similar in both the response to novelty in a highly familiarized environment and the activity in the hole–board apparatus. If this is true, there should be a strong positive correlation between both of these kinds of activity (namely the response to novelty in the free-exploration box and the activity in the hole–board apparatus). Although the validity of the hole–board protocol for novelty-seeking measurement is often assumed implicitly [22], sufficiently robust evidence to support the above claim is still lacking.

In our study, the scores obtained in the hole–board test allowed us to predict the level of rats' activity in the free-exploration box only to a very limited extent. The main factor explaining exploratory responses in the free-exploration box was the environmental change that occurred over the course of the experiment. The factors of sex and HB group designation (which indicated high vs. low hole–board activity scores) were of lower predictive value. The direction of the relation between hole–board activity and exploratory scores in the free-exploration box is similar (e.g., individuals that scored high on the hole–board test manifested a high level of exploratory responses, such as time spent in contact with the tunnels). Moreover, HB-high individuals demonstrated a stronger tendency to spend more time in the modified zone of the experimental chamber. As observed by Žampachová et al. [23], behavioral characteristics measured in an open field and the hole–board test share several common properties. An important characteristic of that study was a repeated measure scheme adopted by the authors. The authors drew conclusions about animal personality rather than any specific mechanisms of exploratory behavior.

Moreover, their arguments overlapped with exploration understood as a mediator of the adaptation process derived from the theoretical framework of behavioral ecology. However, it has little to do with the theoretical speculation about the mechanism of behavior regulation at an individual level. A similar approach was adopted earlier when researchers tested the temperament concept in rats based on the hole–board procedure. J. Ray and S. Hansen [24] tested rats in the hole–board test and the canopy test six times in a 3-week period. They found the hole–board test to be relevant for assessing the rats' temperament; the dimension responsible for exploratory behavior, however, was found to be of secondary importance, after harm avoidance. In a further study [25], they found that the relative role of the two aforementioned dimensions changes with ontogenesis. It seems that the prevalent nature of the dimension directly linked to animals' emotionality, compared to the exploratory (or stimulus seeking) axis, is strictly related to the procedural details, namely the way the animal is placed in the apparatus. The standard procedure involves a human placing the animal in the apparatus's central zone using their hands. This, in turn, may be recognized as a crucial element of the animal's situation, meeting the conditions for what is called a "forced exploration" paradigm. As Márquez, Nadal, and Armario [26] have shown, the hole–board procedure involves some level of stress response in tested animals, especially during the first minutes of measurement. This, in turn, would support the view that the hole–board procedure allows researchers to analyze behavioral measures relevant for "active coping" or reactivity rather than exploration per se. To avoid this obstacle, we decided to use a free-exploration procedural variant, which involved a transportation container which was comfortable for the animal being carried, allowing the animal to stay inside or leave at any moment of the trial into the open area of the hole–board. This was intended to address the main legitimate objection voiced by Hughes [4] and Brown and Nemes [5], who suggested that animal activity may be seen rather as an expression of the tendency to escape/leave the apparatus than to explore it. Indeed, our data do not support the view that the exploratory component of behavior repertoire in the hole–board is predominant. On the contrary, the data seem to support the view expressed by Hughes [4] and Brown and Nemes [5]. The reason for this lies in the ecological validity of the two tests: the standard hole–board apparatus vs. the free-exploration chamber [15]. First, the standard hole–board procedure involves testing under daylight conditions, while our free-exploration box is mainly used in darkness. Studying rats under dark conditions is more ecologically valid, as rat are nocturnal animals and typically avoid brightly light places. Secondly, the hole–board procedure is very rarely combined with several habituation trials, which seem unnecessary and unjustified in the light of the simplicity of the environment that the hole–board offers to the animal. However, novelty results from the discrepancy between the previous experience and actual sensory input. The prolonged habituation allows for control of the selective effect of modified parts of the environment. On the contrary, placing the animal in a completely novel test arena does not allow for the attribution of the behavioral activity manifested by an individual tested to a particular stimulation source. Therefore, it may be easily put forward that novel stimulation's intensity drives an individual to leave the area rather than to explore it. Thirdly, the general structure of the hole–board environment does not offer many affordances. Rather, it offers just one affordance, albeit multiplied.

There is no doubt, however, that the hole–board procedure does measure behavioral responses to a stimulus-rich environment, that it is widely used, and that its reliability is considered sufficient [27]. Nevertheless, one should be cautious when suggesting a theoretical interpretative tool for measuring an animal's activity in the hole–board apparatus. Based on our experience in studying the exploratory behavior of rats in the free-exploration box [15], we conclude that the hole–board apparatus is not an appropriate tool for measuring exploratory behavior in laboratory rodents. However, we believe that the hole–board procedure may be an attractive tool for behavior analysis in many other fields of study (e.g., [28,29]). What is more, additional data obtained from studies with various variants of hole–board and test conditions (e.g., [30]) are needed to propose more

conclusive statements. Nonetheless, this study provides cues for the rethinking of the role of the hole–board procedure as a tool for exploratory activity measurements.

## 5. Conclusions

Based on the results of our study and observations from previous experiments on exploratory behavior in low-stress environments, we must conclude that the hole–board apparatus is not an appropriate tool for measuring exploratory behavior in laboratory rodents. Other behavior regulation mechanisms (e.g., risk assessment, emotional reactivity, active coping) might play a greater role in shaping an animal's activity in the hole–board apparatus. Our results stress the need for cautious reflection on behavioral tests' ecological validity when it comes to the studies on animal behavior.

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# Decrease in the rewarding value of spatial novelty due to the contamination of the stimulus field with light – Evidence from a free exploration test involving rats

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ABSTRACT

It has been shown that rearranging the spatial properties of a familiar environment consistently elicits a positive response in rats directed toward the source of novelty. Previous studies have been conducted under red light or darkness. The purpose of this study was to test the effect of rearranging the spatial properties of a familiar environment in conjunction with a change in lighting conditions. The results have shown specific effects of the light presence and its intensity on different behavioral measures. We propose that this study provides a basis for hypothesizing a two-way mechanism of the behavioral response to light regulation in rats. The first is based on ON/OFF states. This level may be related to fundamental, evolutionarily early, emergent components of behavioral antipredator adaptations. Another level of behavioral regulation involves mechanisms sensitive to light intensity. These appear to be involved in the regulation of more advanced behavioral acts, such as exploratory responses. This may suggest that light intensity analysis may require the involvement of more advanced cognitive components in the behavioral regulation system.

## 1. Introduction

Rearrangement of the spatial properties of familiar surroundings, conducted in low-stress conditions, has been consistently shown to produce a positive response directed towards the source of novelty (Pisula, 2003; Pisula, 2004; Pisula et al., 2019; Pisula et al., 2021), an effect which is characterized by a high degree of repeatability. Standardizing the testing procedure (Pisula and Modlinska, 2020) allowed us to compare the results across multiple studies. To date, our experiments have been conducted using red light (Pisula, 2003; Pisula, 2004) or in darkness (Pisula et al., 2019; Pisula et al., 2021). The goal was to expose the animals to the lowest possible level of stress when conducting the measurements. Moreover, it opened up a new possibility of controlling the potential sources of stressful/aversive stimulation, such as illumination of the test arena. The present study is a continuation of our long-standing work on the mechanisms of exploratory behavior regulation in rats in low-stress laboratory conditions. The purpose of the study was to examine the effect of the rearrangement of the spatial properties of familiar surroundings in combination with changes in the lighting conditions, which may be labeled 'contamination of the stimulus field with light'.

The aversive/nociceptive role of light in rat behavior regulation has long been the focus of scientific inquiry. As early as F. Keller, 1941 demonstrated that increased light intensity might produce aversive reactions, manifested by increased lever-pressing behavior rewarded by the lights being turned off. These findings were followed by the results obtained by Stellar, Hunt, Sch losberg and Solomon (Stellar et al., 1952). Those researchers demonstrated that hoarding behavior in rats correlates positively with light intensity. Compatible results were obtained (Valle, 1970) in the open-field test. High-intensity light produced a decrease in ambulation and rearing in rats. The aversive aspect of exposure to white light has been widely documented also in more recent studies on anxiety and fear in rats (e.g. (Bouwknecht et al., 2007; Garcia et al., 2005; Hale et al., 2008; Kuniishi et al., 2017). The authors were able to show that rats explore an unfamiliar T-maze under no-light (0 lx) or very low-light (1 lx) conditions much more intensively than in setups involving higher light intensity (starting at 3 lx). This is one of the findings demonstrating the inhibitory aspect of white light stimulation linked to exploratory behavior in rats. Another important conclusion may be neatly summarized with a quote from (Campbell and Messing,

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1969): '[p]erhaps the most striking feature of the present study was the failure to find any intensity of light that was preferred by rats of either strain significantly more than total darkness'. While light of high intensity (more than 65–70 lx) may harm the rats' retina, especially in Albinotic individuals (for an overview, see (Burn, 2008), stimulation with white light has also been shown to produce a rewarding effect. This was demonstrated as early as 1955 (Kish, 1955) in a study on mice which learned to press a bar when the 'lights-on' setting was used as a reinforcing stimulus. This phenomenon was labeled 'sensory reinforcement' (Barnes et al., 1959; Kish, 1955) and became an important explanatory tool for such behavioral phenomena as stimulus-seeking (Pisula et al., 1992; Gancarz et al., 2012) and exploration (c.f. (Pisula, 2020). Therefore, depending on the circumstances, stimulation with white light may play either an appetitive or an aversive role. It should be noted here that we are discussing the properties of light stimulation as such, and not its ability to induce rats to form associations with another stimulus (either aversive or appetitive). Perhaps controlling changes in the stimulus is the most important element in such settings, as summed up by G.B. (Kish, 1955): '[a] perceptible environmental change, which is unrelated to such need states as hunger and thirst, will reinforce any response that it follows'.

Due to the complex nature of white light stimulation in rat behavior regulation, we decided to include this source of environmental complexity in our study. We wanted to incorporate the element of light manipulation into the sensory/perceptual field of the rats' environment while preserving the low-stress properties of the test arena. Therefore, we used two setups with a dimmed white light of low intensity. In most studies, the level of light intensity applied by the researchers was high (e.g. (Godsil and Fanselow, 2004). Although the authors did not provide information on the level of light intensity obtained from three 100 W bulbs from an 18-cm distance, our estimation would be that it reached at least 200–300 lx. In the present study, the animals may have experienced the maximum light intensity of 65 lx only in close contact with the light source (see the 'Procedure' section for details).

We expected that if the light does in fact evoke the inherent aversive response in rats, then both low-level intensity settings would inhibit the exploration of recently modified tunnels, even if the intensity of light was low in both cases. On the other hand, if the aversive property of light stimulation is a function of light intensity, we would obtain a differentiated light impact profile, depending on light intensity.

## 2. Material and methods

### 2.1. Animals

The sample consisted of 38 male Lister Hooded rats. The rats were bred and housed in the vivarium of the Institute of Psychology, Polish Academy of Sciences, Warsaw, Poland. At the beginning of the study, the rats were approx. 90 days old and weighed approx. 350 g.

The rats were housed in groups of 3–4 in Tecniplast© Eurostandard Type IV cages (610 mm × 435 mm×215 mm) with dust-free softwood granules Tierwohl Super© as bedding. They had ad libitum access to water and standard laboratory fodder (Labofeed H, WP Morawski, Kcynia, Poland). The day/night cycle was set at 12/12 h (lights-on at 8.00 a.m.). The light intensity in the home cages ranged from 80 lx at the back of the cage to the approx. 380 lx in the front. The temperature was maintained at a constant 21–23 °C, and humidity at 45–60%. Prior to the experiment, the cages were cleaned once a week. However, in order to ensure that the experimental procedure was not disturbed, the cages in which the test animals were kept were cleaned just before the start of the behavioral test and again after the test was completed.

All the rats were housed, bred and taken care of in accordance with the Regulation of the Polish Minister for Agriculture and Rural Development of 14 December 2016 on laboratory animal care. The experimental procedures had been approved by the First Local Committee for Ethics in Animal Experimentation in Warsaw, Poland.

The sample size was estimated using a commonly used formula for calculating sample size for repeated measures recommended by the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (Institute for Laboratory Animal Research (U.S.), National Academies Press (U.S.), 2003). The minimal sample size for the Exploration test was estimated at 10.

### 2.2. Procedure

The purpose of the exploration test was to compare the process of exploring a new environment, the pace of habituation to it, and the responsiveness to the introduction of a low-intensity innocuous novelty in a familiar setting. The apparatus and the methods of measurement were identical to those used in our previous studies (Pisula and Modlinska, 2020).

The experimental chamber (Fig. 1) was a box measuring 800 mm × 600 mm× 800 mm. The chamber was divided into three zones (A, B, C) by two walls running perpendicularly to its longer side. The partition walls between the zones had triangular openings (120 mm × 140 mm) at the bottom, which enabled free movement between the chamber parts. There was a hole curved in the back wall of the chamber that served as an entrance for animals moving from the transporting device into the chamber. The front of the chamber was made of a transparent plexiglass and it could be lifted to obtain full access to the experimental arena. The entire chamber was covered with a layer of washable varnish. There were tunnels (200 mm × 120 mm×80 mm) placed in zones B and C made of hard wood covered with washable paint. In contrast to the most frequently used two-dimensional experimental settings, these tunnels provide a complex three-dimensional environment. The central zone (A) was left empty.

At the start of each trial, a small cylindrical cage (the 'transporter' – 60 mm in diameter with doors 120 mm high and 100 mm wide) with the tested animal inside was placed by the entrance to zone A. The entrance door was then lifted and it was left open until the end of the trial. The animal was free to stay in the transporter or leave it to explore the chamber. The first seven trials were habituation trials during which the apparatus was arranged in the same way. The introduction of novelty (addition of novel tunnels, - see Fig. 2) took place before the T1 trial, that is between trials 7 and 8. The three subsequent trials were conducted with the chamber in this new arrangement (Fig. 2). Each trial was 7 min long and was conducted for each animal once a day. Measurements were conducted during the light phase of the day/night cycle. It started at

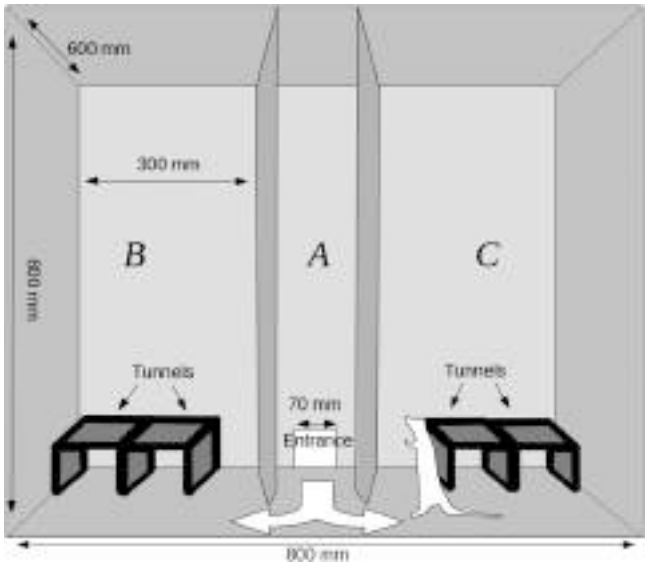


**Fig. 1.** Experimental chamber - frontal view through the transparent front wall.

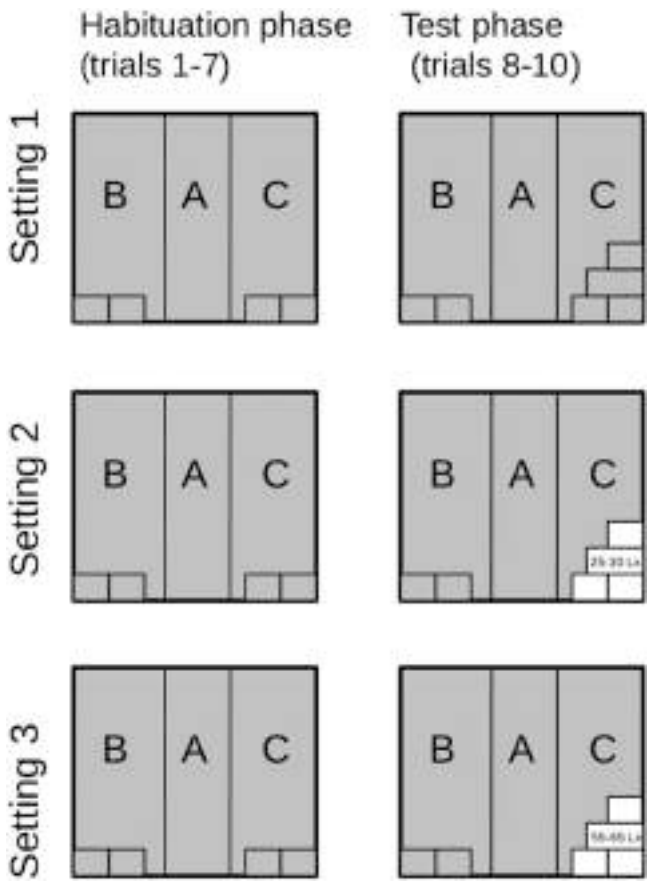


**Fig. 2.** Arrangement of objects and light conditions in the experimental chamber in each experimental setting.

10 am (the second hour of the cycle) and ended at about 2 pm.

Three series of tests were conducted which differed with regard to the configuration of the tunnels that were placed in the experimental chamber, as well as the type of novelty introduced in the 8th trial. In each experimental setting, the tunnels were placed in zones B and C.

Setting 1 - Addition of a novel object to the other objects in the experimental box. During the habituation sessions, two tunnels (200 mm × 120 mm×80 mm) had been placed in each of the zones B and C and arranged in the same way (Fig. 1). On the first test trial day (trial 8), two additional tunnels were put in zone C (Fig. 2). The arrangement of the tunnels in zone B remained unchanged. This setting was described as the 'NoLight' group. This manipulation is a direct replication of the 2019 study. The setting was explored by a sample of 10 rats.

Setting 2 - Same spatial manipulation as in the 'NoLight' group. However, the back wall of the chamber emitted dimmed white light of 25–30 lx intensity (measured at a 10 cm distance from the illuminated wall), evenly distributed within the outline of the tunnels (Fig. 2). We labeled this setting the 'LowLight' setup. This setup was explored by a sample of 14 rats.

Setting 3 - Same spatial manipulation as in the 'LowLight' and 'NoLight' groups. However, the back wall of the chamber emitted dimmed white light of 55–65 lx intensity (measured at a 10 cm distance from the illuminated wall) (Fig. 2). We labeled this setting 'HighLight'. This setting was explored by a sample of 14 rats.

To avoid the confounding effect of lateralization or visual/auditory cues, the novelty was introduced in the left zone of the experimental chamber as described above for half of the rats tested, and in the right zone of the experimental chamber for the remaining half (a mirror image of Fig. 2).

### 2.3. Data processing and statistical analyses

To encode the behaviors on the basis of the recorded material we used BORIS software (Friard and Gamba, 2016), which made it possible to define selected behaviors and assess their duration and frequency. We scored the behaviors the animals engaged in during the entire experimental trial. Consequently, we could assign specific scores to the duration of separate bouts of behaviors, their frequency, and the total time an animal spent engaging in a given behavior. The following variables were measured: (1) Time spent in the transporter (excluding the latency to leave the transporter); (2) Time spent in the central zone; (3) Time spent in the unchanged zone of the chamber; (4) Time spent in the changed zone of the chamber; (5) Frequency of moving between the zones (left/right/transporter) of the chamber; (6) Time spent on contact with the tunnels in the unchanged zone of the chamber; (7) Frequency of contact with the tunnels in the unchanged zone of the chamber; (8) Time spent on contact with the tunnels in the changed zone of the chamber; and (9) Frequency of contact with the tunnels in the changed zone of the chamber.

The next step was establishing a reference value for the study samples. To do this, the last three habituation trials (H5 to H7) were aggregated, and means for all subjects/variables were calculated. These values were then labelled as trial H values. Since the main focus of this study was on the animal response to novelty and the habituation phase served only as a baseline, we transformed the scores of the habituation phase into Z-scores to emphasize the changes occurring after the introduction of novelty. The H-values were converted to Z scores within the three experimental settings separately. Therefore, within all experimental trials, the sample means H values were set to "0." Then, all values from trials T1 through T3 were converted to Z scores based on the habituation trial H statistics (mean, StdDev, before conversion to Z). To enhance the legibility of the results and tables, the habituation phase has been indicated as H, while the three test trials have been indicated as T1, T2, and T3, respectively.

We have decided not to present the results of the initial four habituation trials since they serve only as the habituation phase and not as an element of a comparative analysis of the animals' response to novelty.

These data were then used in a Repeated Measures ANOVA analysis with (H, T1, T2, T3) as within-subject factor Trial, as well as group assignment (NoLight, LowLight, HighLight) as between-subject factor LightCond. Greenhouse-Geisser Sphericity Correction was used whenever the sphericity assumption was violated. This was followed by PostHoc t-tests with Bonferroni correction for multiple comparisons. Differences were considered significant for $p \leq 0.05$. Since the experimental design applied in this study was based on repeated measures, our main focus was on the interaction between Trial and LightCond effects and not on the simple effects of these factors taken separately. If an interaction effect was found, a posthoc analysis would be limited to breaking down the interaction effect.

## 3. Results

### 3.1. Time spent in the transporter

The analysis showed the main effect of Trial: $F(2.4, 84.015) = 7.700$, $p < 0.001$, $eta2 = 0.180$. A post hoc analysis showed a significant decrease in the time spent in the transporter in the first test trial T1 ($t(37) = 4.763$, Cohen's $d = 0.773$, $p < 0.001$), and the second trial T2 ($t$

(37) = 2.916; Cohen's d= 0.473, p = 0.026), as compared to the habituation phase (H), but not in the third test trial T3 t(37) = 2.406, p = 0.107.

### 3.2. Time spent in the unchanged zone of the chamber

There were no effects of experimental manipulations on the amount of time spent in the unchanged zone of the chamber.

### 3.3. Time spent in the changed zone of the chamber

There was a main effect of Trial: F(3, 105)= 13.696, p < 0.001, eta2 = 0.281. A post hoc analysis showed a significant increase in the time spent in the changed zone of the chamber in all test trials (T1, T2, T3) compared to the habituation trial [T1: ($t$(37) = −5.654, Cohen's d= −0.917, p < 0.001); T2: ($t$(37) = −3.995, Cohen's d= −0.648, p < 0.001); T3: ($t$(37) = −5.423, Cohen's d= −0.880, p < 0.001)].

### 3.4. Time spent in the central zone of the chamber

The analysis showed the main effect of Trial [F(2.324, 81.357)= 3.721, p = 0.023, eta2 = 0.096], as well as an interaction (Trial x LightCond) effect on the time spent in the central zone of the chamber [F (4.649, 81.357)= 2.602, p = 0.034, eta2 = 0.129]. Subjects from the LowLight group showed a significant decrease in that time when trials T2 and T3 were compared ($t$(13) = 4.316, Cohen's d= 0.656, p = 0.002). However, there were no significant differences between habituation phase (H) and trial T1 ($t$(13) = 1.889, p = 1.000) and between trials T1 and T2 ($t$(13) = 2.824, p = 0.375) in LowLight group.

### 3.5. Frequency of moving between the chamber zones (left/right/transporter)

The main effect of Trial was found [F(3105)= 4.143, p = 0.008, eta2 = 0.106]. A post hoc analysis showed a significant decrease in the frequency of moving between the chamber zones when habituation trial (H) and T3 were compared ($t$(37) = 3.365, Cohen's d= 0.546, p = 0.006). However, there were no significant differences between habituation phase (H) and trial T1 ($t$(37) = 1.397, p = 0.992), between trials T1 and T2 ($t$(37) = 1.005, p = 1.000) and between trials T2 and T3 ($t$(37) = 0.963, p = 1.000).

### 3.6. Frequency of contact with the tunnels in the unchanged zone of the chamber

The RM ANOVA showed the main effect of the Trial factor [F(2.291, 80.184)= 4.596, p = 0.010, Et2 = 0.116] on the frequency of contact with the tunnels in the unchanged zone of the chamber. The analysis also detected an interaction (Trial x LightCond) effect [F(4.582, 80.184)= 2.502, p = 0.041, eta2 = 0.125]. A post hoc analysis showed a significant decrease in the frequency of contact with the tunnels in trials T2 ($t$(13) = 3.499, Cohen's d= 1.159, p = 0.045) and in T3 ($t$(13) = 3.896, Cohen's d= 1.186, p = 0.011) compared to the habituation phase (H) in NoLight rats, but not between habituation phase (H) and trial T1 ($t$(13) = 0.924, p = 1.000), between trials T1 and T2 ($t$(13) = 2.575, p = 0.753) or between trials T2 and T3 ($t$(13) = 0.396, p = 1.000). Rats from either of the groups exposed to light manipulations did not manifest this effect. However, rats from the HighLight group interacted with the tunnels more than the NoLight subjects in trial T2 ($t$(26) = 3.719, Cohen's d= 1.371, p = 0.021), but not in trial T1 ($t$(26) = 1.800, p = 1.000) or in trial T3 ($t$(26) = 3.419, p = 0.059).

### 3.7. Time spent on contact with the tunnels in the unchanged zone of the chamber

There were no effects of experimental manipulations on the amount of time spent in contact with the tunnels in the unchanged zone of the chamber.

### 3.8. Frequency of contact with the tunnels in the changed zone of the chamber

There were no effects of experimental manipulations on the amount of time spent on contact with the tunnels in the changed zone of the chamber.

### 3.9. Time spent on contact with the tunnels in the changed zone of the chamber

The analysis showed the main effect of Trial [F(3, 105)= 21.264, p < 0.001, eta2 = 0.378], as well as an interaction (Trial x LightCond) effect on time spent on contact with the tunnels in the changed zone of the chamber [F(6, 105)= 3.879, p = 0.002, eta2 = 0.181].

Since a post hoc analysis delivered many effects, they will be shown in a tabular form in Table 1.

Additionally, rats from the HighLight group interacted with the tunnels in unchanged zone less than the NoLight subjects in trial T1 ($t$ (26) = 4.170, Cohen's d= 2.204, p = 0.004), and in trial T3 ($t$(26) = 4.277, Cohen's d= 1.354, p = 0.003), but not in trial T2 ($t$(26) = 1.065, p = 1.000).

### 3.10. Effect size analysis

Taking as our starting point the 2019 study (Pisula et al., 2019), we analyzed the effect size coefficients on multiple occasions. The partial Eta2 allows for comparing both effects obtained in different studies and the results obtained in the same study but various analyses. The latter one is how we have used this method since the 2019 study. The Eta2 values obtained in this study are summarized in Table 2. A Kruskal-Wallis ANOVA showed no significant difference in Eta2 between the experimental factors.

Descriptive statistics of all variables that were measured in this study are shown in the table provided in Appendix 1.

**Table 1**
Post Hoc (Bonferroni) Comparisons - LightCond ✻ Trial. Cohen's d calculated only for statistically significant effects.

| Condition | Comparison (trials or groups) | Mean Difference | t | df | p | Cohen's d |
|---|---|---|---|---|---|---|
| HighLight | H vs T1 | -0.667 | -0.868 | 13 | 1.000 | – |
| | H vs T2 | -1.723 | -2.240 | 13 | 1.000 | – |
| | H vs T3 | -1.416 | -1.841 | 13 | 1.000 | – |
| | T1 vs T2 | -1.056 | -1.373 | 13 | 1.000 | – |
| | T1 vs T3 | -0.749 | -0.973 | 13 | 1.000 | – |
| | T2 vs T3 | 0.307 | 0.399 | 13 | 1.000 | – |
| LowLight | H vs T1 | -3.468 | -4.510 | 13 | < 0.001 | 1.359 |
| | H vs T2 | -2.761 | -3.591 | 13 | 0.033 | 0.986 |
| | H vs T3 | -3.064 | -3.985 | 13 | 0.008 | 1.038 |
| | T1 vs T2 | 0.706 | 0.918 | 13 | 1.000 | – |
| | T1 vs T3 | 0.403 | 0.525 | 13 | 1.000 | – |
| | T2 vs T3 | 0.303 | 0.394 | 13 | 1.000 | – |
| NoLight | H vs T1 | -4.975 | -5.468 | 13 | < 0.001 | 2.369 |
| | H vs T2 | -2.823 | -3.103 | 13 | 0.163 | – |
| | H vs T3 | -5.834 | -6.412 | 13 | < 0.001 | 1.545 |
| | T1 vs T2 | 2.152 | 2.365 | 13 | 1.000 | – |
| | T1 vs T3 | -0.859 | -0.944 | 13 | 1.000 | – |
| | T2 vs T3 | -3.011 | -3.309 | 13 | 0.085 | – |

Note. p-value adjusted for comparing a family of 66.

**Table 2**
Eta2 coefficients of all possible experimental effects in this study. Eta2 of statistically non-significant effects are set to '0'.

| Dependent variables | Exp. Factor | Eta2 |
|---|---|---|
| Time spent on contact with the tunnels in the changed zone of the chamber | trial | 0.378 |
| Time spent in the changed zone of the chamber | trial | 0.281 |
| Time spent on contact with the tunnels in the changed zone of the chamber | trial x light Cond | 0.181 |
| Time spent in the transporter | trial | 0.180 |
| Time spent in the central zone of the chamber | trial x light Cond | 0.129 |
| Frequency of contact with the tunnels in the unchanged zone of the chamber | trial x light Cond | 0.125 |
| Frequency of contact with the tunnels in the unchanged zone of the chamber | trial | 0.116 |
| Frequency of moving between the chamber zones (left/right/transporter) | trial | 0.106 |
| Time spent in the central zone of the chamber | trial | 0.096 |
| Frequency of contact with the tunnels in the changed zone of the chamber | trial | 0 |
| Frequency of contact with the tunnels in the changed zone of the chamber | trial x light Cond | 0 |
| Frequency of moving between the chamber zones (left/right/transporter) | trial x light Cond | 0 |
| Time spent in the changed zone of the chamber | trial x light Cond | 0 |
| Time spent in the transporter | trial x light Cond | 0 |
| Time spent in the unchanged zone of the chamber | trial | 0 |
| Time spent in the unchanged zone of the chamber | trial x light Cond | 0 |
| Time spent on contact with the tunnels in the unchanged zone of the chamber | trial | 0 |
| Time spent on contact with the tunnels in the unchanged zone of the chamber | trial x light Cond | 0 |

### *3.11. Summary of the results*

The rats in our study responded to novelty by spending more time in the modified zone of the chamber. This was linked to a decrease in the amount of time spent in the central zone and transporter, especially in the LowLight group. There were no changes in the duration of staying in the unchanged zone of the chamber.

All rats moved less frequently between the chamber zones, which may be viewed as a manifestation of the habituation process.

The rats from the NoLight setting also showed an expected decrease in the frequency of interactions with the tunnels in the unchanged zone of the chamber. However, the two other groups did not exhibit this pattern. The rats' interactions with the tunnels in the changed zone of the chamber painted a different picture of behavioral responses to novelty. The rats from the NoLight and LowLight settings manifested an expected increase in the duration of interaction with the tunnels across trials T1 to T3, compared with the habituation trial (H). The rats from the HighLight conditions did not show this effect.

## 4. Discussion

The general pattern of behavior in this study resembled the findings from our previous experiments (Pisula et al., 2019; Pisula et al., 2021). All rats responded to the rearrangement of the tunnels by spending more time in the modified zone of the chamber. This recurring response pattern to novelty was slightly modified in the present study by introducing the element of exposure to white light (low-intensity light stimulus). Nevertheless, exposure to dimmed white light affected the rats' behavior in the free exploration test arena. This was observed both in the unchanged and in the changed zones of the experimental chamber. If the light is to have inherent intensity-independent aversive properties for rats, the patterns of behavioral response in the two intensity setups should be similar. The results obtained in our study, however, suggest a more complex picture. All rats modified their time budget by allocating more time to staying in the chamber zone with the changed tunnels. This may result from a shift in preferences towards staying in the more complex, and therefore more attractive, modified/changed zone of the chamber. The time budget allocation for staying in the individual zones of the chamber was affected by the light conditions. This was the case in the LowLight group in the central zone of the chamber. However, rats from neither experimental group reduced the amount of time spent in the unchanged zone of the chamber. This may be interpreted as an indication of the relative attractiveness of the dark, albeit not modified, zone of the chamber. This behavioral pattern seems compatible with the results of obtained by Farnworth et al. (2019). The authors found that ambient illumination is a factor resulting in less time being spent in semi-natural testing conditions. The temporal structure of the rats' visits to the test arena was also affected by lighting. The results were discussed in terms of anti-predatory tactics in rats. These theoretical tools seem applicable to our study as well. It may be argued that there are some species-specific behavior regulation mechanisms linked to light avoidance in rats, triggered by the LIGHTS ON / LIGHT OFF settings rather than by (subtle) changes in light intensity.

However, we also observed some behavioral responses specific to light intensity levels. The rats tested in the NoLight conditions showed an expected decline in the frequency of interactions with tunnels in the unchanged zone of the chamber, which is an effect already observed in our previous studies (Pisula et al., 2019; Pisula et al., 2021). However, the two other groups did not exhibit this pattern. This deviation from the previously established response pattern may be discussed regarding environmental complexity. Rats subjected to environmental change or a higher degree of complexity maintained the preference for the unchanged, stable zone of the chamber throughout all trials. This may reflect an action of regulatory mechanisms maintaining the level of complexity of incoming stimulation at a relatively stable level. In a similar vein, the animals from the NoLight and LowLight groups showed an expected increase in the amount of time spent on interaction with the tunnels across trials T1 to T3 in the modified chamber zone. These results demonstrate an increase in the attractiveness of the more complex environment. However, the rats from the HighLight conditions did not exhibit this effect. They manifested a complex response involving a prolonged stay in the zone with the modified tunnels but no close contact with the tunnels. One may hypothesize that this reflects a classic approach-withdrawal conflict that kept animals close to the modified tunnels but prevented them from touching the tunnels and exploring them in any depth. We may conclude therefore that the intensity of light, even where it remains within a low-intensity range and is tested in low-stress conditions, remains an important aspect of stimulation. Since both the intensities of the light stimulation applied in our study should be considered as low or moderate, the unconditioned anxiety-evoking properties of such stimulation seem not to be the main variable explaining the obtained phenomenon. Light avoidance in rats is a highly modifiable phenomenon. Rats adjust their daily cycle of activity to the ecological conditions. Classic studies on sensory reinforcement (Kish, 1955) proved that light may play a role of reward. Therefore the results of the differentiated response to light intensities investigated in our study direct our attention to the cognitive aspects rat coping with the environmental change.

Generally, this tallies with the results of experiments conducted, e.g. by Garcia et al. (2005). However, in many previous studies, the experimental setup did not provide as comfortable and low-stress conditions as our procedure. The procedure we used, described in detail by Pisula and Modlinska (2020), had been designed to induce as little stress as possible in the animals tested. This was achieved mainly by the long habituation phase, ensuring a high level of familiarization with the test environment. It seems that in these low-stress conditions, it is possible to distinguish two distinct behavioral responses, dependent on both the LIGHTS ON / LIGHTS OFF dichotomy and changes in light intensity.

## 5. Conclusions

We believe that this study provides grounds for hypothesizing the existence of two-way mechanisms underlying the behavioral response to light regulation in rats. The first one relies on the LIGHTS ON / LIGHTS OFF dichotomy. At first glance, this resembles the position taken by Saito and Brandão (2016), who interpreted light avoidance as a measure of innate rodent fear of bright-lighten areas. This level might be associated with more fundamental, evolutionary early-emergent components of anti-predatory behavioral adaptations.

Another level of behavior regulation involves mechanisms that are responsive to light. They seem to be involved in the regulation of more advanced behavioral acts such as exploratory responses, which in our study was reflected by such measures as frequency and amount of time spent on contact with the tunnels. This, in turn, may suggest that an analysis of light intensity may require taking into consideration more advanced cognitive components of the behavior regulation system. This theoretical proposal must be tested further in experimental studies.

## CRediT authorship contribution statement

**Wojciech Pisula:** Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **Klaudia Modlinska:** Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing. **Anna Chrzanowska:** Funding acquisition, Investigation, Writing – original draft. **Katarzyna Goncikowska:** Investigation, Writing – original draft. All authors reviewed and accepted the manuscript.

## Competing interests

The authors declare no competing interests.

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## Appendix 1. Descriptive statistics of all variables measured in the study

| Trial | H | | T1 | | T2 | | T3 | |
|---|---|---|---|---|---|---|---|---|
| Condition | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| | Frequency of moving between the chamber zones (left/right/transporter) | | | | | | | |
| HighLight | 18.119 | 6.136 | 18.429 | 3.567 | 17.214 | 4.644 | 17.357 | 5.472 |
| LowLight | 20.833 | 6.115 | 18.357 | 2.898 | 18.500 | 5.346 | 17.929 | 5.784 |
| NoLight | 19.167 | 5.580 | 18.400 | 5.379 | 17.400 | 5.400 | 15.900 | 3.814 |
| | Time spent in the central zone of the chamber | | | | | | | |
| HighLight | 112.301 | 22.472 | 113.565 | 24.402 | 104.952 | 32.293 | 112.556 | 30.980 |
| LowLight | 120.894 | 20.897 | 102.962 | 21.761 | 129.763 | 52.345 | 88.797 | 28.656 |
| NoLight | 111.220 | 23.513 | 93.405 | 24.182 | 99.962 | 28.381 | 88.135 | 18.746 |
| | Frequency of contact with the tunnels in the changed zone of the chamber | | | | | | | |
| HighLight | 6.810 | 2.653 | 6.357 | 3.079 | 6.357 | 3.003 | 6.571 | 3.275 |
| LowLight | 7.357 | 2.458 | 8.857 | 2.413 | 7.357 | 2.678 | 7.429 | 2.766 |
| NoLight | 6.467 | 2.520 | 7.400 | 2.675 | 7.500 | 3.749 | 7.100 | 2.424 |
| | Time spent on contact with the tunnels in the changed zone of the chamber | | | | | | | |
| HighLight | 67.907 | 22.197 | 82.716 | 44.884 | 106.147 | 65.922 | 99.332 | 59.397 |
| LowLight | 63.224 | 18.711 | 128.110 | 42.394 | 114.894 | 49.808 | 120.560 | 54.697 |
| NoLight | 57.454 | 12.030 | 117.301 | 22.281 | 91.413 | 46.325 | 127.638 | 47.675 |
| | Time spent in the changed zone of the chamber | | | | | | | |
| HighLight | 113.222 | 24.347 | 128.389 | 58.668 | 143.477 | 69.743 | 138.611 | 71.299 |
| LowLight | 108.190 | 28.793 | 184.488 | 45.408 | 150.414 | 56.239 | 161.265 | 67.928 |
| NoLight | 95.583 | 18.420 | 156.315 | 25.387 | 131.199 | 58.507 | 158.488 | 52.691 |
| | Time spent in the transporter | | | | | | | |
| HighLight | 86.575 | 32.166 | 62.005 | 53.533 | 58.194 | 31.110 | 75.975 | 58.856 |
| LowLight | 89.068 | 42.859 | 42.822 | 19.128 | 55.324 | 38.544 | 78.414 | 64.952 |
| NoLight | 100.778 | 22.356 | 63.285 | 18.814 | 89.930 | 64.004 | 73.953 | 43.551 |
| | Frequency of contact with the tunnels in the unchanged zone of the chamber | | | | | | | |
| HighLight | 6.167 | 2.331 | 7.357 | 2.307 | 6.929 | 2.200 | 6.143 | 2.381 |
| LowLight | 6.500 | 2.297 | 6.929 | 1.639 | 6.143 | 2.770 | 6.143 | 2.413 |
| NoLight | 6.867 | 1.102 | 6.400 | 2.591 | 5.100 | 2.079 | 4.900 | 1.853 |
| | Time spent on contact with the tunnels in the unchanged zone of the chamber | | | | | | | |
| HighLight | 62.264 | 20.779 | 73.898 | 30.086 | 72.305 | 19.843 | 57.341 | 38.569 |
| LowLight | 57.130 | 11.439 | 49.249 | 18.989 | 46.031 | 17.089 | 53.664 | 16.875 |
| NoLight | 65.671 | 17.119 | 65.777 | 17.048 | 63.405 | 36.236 | 58.228 | 23.179 |
| | Time spent in the unchanged zone of the chamber | | | | | | | |
| HighLight | 102.904 | 30.144 | 113.509 | 40.960 | 111.524 | 30.508 | 91.421 | 52.088 |
| LowLight | 99.806 | 15.980 | 88.024 | 28.877 | 83.100 | 37.209 | 89.992 | 27.594 |
| NoLight | 109.932 | 18.898 | 104.007 | 19.923 | 97.401 | 41.542 | 97.612 | 38.241 |

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# Response to Perceptual Novelty in Tortoises-A Preliminary Study

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**Abstract**

The ways of responding to novelty have not yet been thoroughly and comprehensively researched in reptiles, and studies undertaken so far have been conducted mainly on laboratory rats. The purpose of this paper is to present results of research carried out on the land tortoises *Testudo hermanni* and *Agrionemys horsfieldii*. Current research on tortoises as study subjects indicates that while undertaking environmental exploration these animals rely, to a large extent, on their sight, which is why this study employed visual stimuli to measure the tortoises' response to novelty. In the course of the experiment, three objects were

presented: the first imitated a biologically significant stimulus, the second was biologically indifferent, while the third was variable, and therefore perceived by the animals as novel. The results obtained suggest that the biologically significant and the variable objects elicited a response which involved the animals' looking more intently at those objects than at the biologically indifferent object. It may be concluded, therefore, that tortoises have demonstrated exploratory responses which varied according to the object presented. While this study was only preliminary, the results obtained substantiate the view that tortoises may be interesting study subjects in comparative psychology.

**Keywords:** Exploratory activities, Response to novelty, Land tortoises, Amniotes

## 1. Introduction

Response to novelty is a behavioural element classified as exploratory activity. Research on exploratory behaviour aims to understand the process of organisms' adaptation to environmental changes. Exploration involves collecting environmental information (Pisula, 2009). It is assumed that novelty has a rewarding value for study subjects (Pisula, 2009).

Tortoises' ability to learn has long been the focus of various studies (e.g. Tinklepaugh, 1932). Researchers, however, have not, as yet, demonstrated particular interest in examining the ways tortoises deal with novelty. Studies investigating the ways of dealing with novelty have been conducted on laboratory animals, mainly rats (Pisula, 2009). Nonetheless, many researchers have presented evidence on common anatomical brain features in species belonging to different classes of amniotes (Broglio et al. 2015; Salas, Broglio and Rodríguez, 2003). For these reasons, it seems interesting to carry out comparative studies of species form various classes of animals. Such an approach may help clarify the evolution of specific higher psychological functions in amniotes.

Current research on tortoises as study subjects indicates that while undertaking environmental exploration, these animals rely, to a large extent, on their sight. Recent study suggest that Hermann's tortoises differentiate between colours of both naturally occurring flowers and artificial objects (Pellitteri-Rosa, Sacchi, Galeotti, Marchesi and Fasola, 2010). Tortoises were presented with the following colours: blue, white, red and yellow, as well as with natural flowers of different colours, e.g. bright yellow or bright purple. The animals were most successful in discerning yellow, but they were able to differentiate between the other colours as well (Pellitteri-Rosa et al., 2010).

The presented study was conducted on *T. hermanni* and *A. horsfieldii*. Both species belong to herbivores. In their natural habitat, Hermann's tortoises (*Testudo hermanni*) inhabit rocky grass-covered areas with high sunlight exposure. Their natural habitat covers the area of the Mediterranean Basin. Hermann's tortoises are active in spring, summer and autumn, while they hibernate in winter. The peak of their circadian activity is the morning and the late afternoon. While the Central-Asian tortoises (*Agrionemys horsfieldii*) inhabit dry rocky grass-covered areas. They are active in spring, and may hibernate for as long as nine months in a year. In their natural habitat, plant food is available for approx. 3 months only, which is the reason why their life cycle is heavily dependent on the vegetation season. Central-Asian

tortoises are active for approximately 7 hours a day. Their diet is rich in fibre, but poor in protein (Bergmann, 2001). The naturally occurring populations are becoming extinct due to mass-scale harvesting of this species (Stubbs, 1989).

The nomadic lifestyle of these tortoise species and their opportunistic feeding habits may substantiate a thesis that the novelty of the stimulus plays a significant regulatory role in shaping the behaviour of these animals. As the importance of sight in regulating their behaviour is well-documented (Pellitteri-Rosa et al., 2010), the experiments described below involved the use of visual stimuli.

The purpose of our study was to investigate the responses of land tortoises to new objects which appeared in their immediate environment.

## 2. Method

### *2.1 Animals Studied*

Study subjects were three experimentally naive tortoises: two of the *Testudo hermanni* species (females) and one of the *Agrionemys horsfieldii* species (male). At the start of the experiment, the *Testudo hermanni* tortoises were approx. 15 and 13 years old. The *Agrionemys horsfieldii* was approx. 14 years old. Prior to the experiment, they dwelled in pens (77x70x20 cm): the *Testudo hermanni* communally in one pen, and the *Agrionemys horsfieldii* solitarily in another pen. The tortoises had constant access to water. For 12 hours a day they were exposed to an additional heat source – a standard 60 WATT light bulb placed in the corner of each of the pens. The temperature inside the pens was approx. 23 degrees Celsius, and the humidity level was maintained at approx. 50%. All of the tortoises participating in the experiment had been hibernating for a period of 5 months, during the winter time (November-March). The experiment was conducted in the course of July and August.

### *2.2 Research Apparatus*

The study space comprised an experimental arena surrounded with a 80-cm-tall screen to prevent the animals from looking at any objects outside of the arena (see Fig. 1). A dome-shaped box (30 cm in diameter, 11 cm in height) with three symmetrically distributed openings (2.4 cm in diameter) was placed in the middle of the arena. The size of the openings made it possible for the tortoises to stick their heads out of the openings without encountering any physical barrier. Three open tunnels (15 cm wide, 15 cm high and 30 cm long) were fixed at the level of the openings. The dome-shaped box was placed in the same position during each study session and it was immobilised by means of an additional weight to avoid displacement during the experiment.

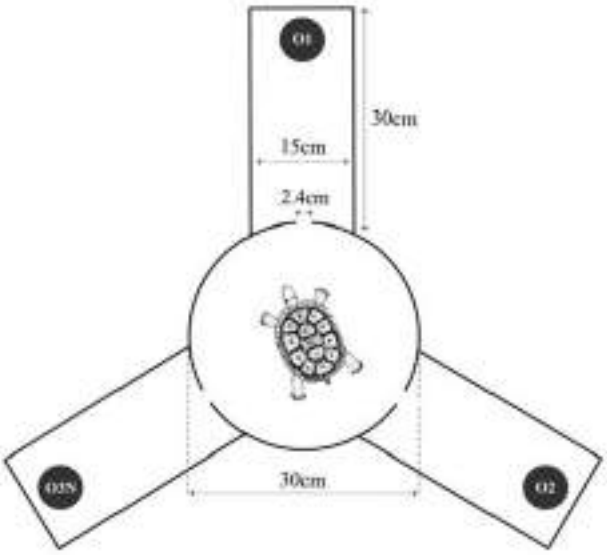


Fig. 1. An outline of the experimental arena. O1 – biologically significant object, O2 – biologically insignificant object, O3N – stimulus variable between sessions.

The objects (O1, O2, O3N) were placed at the end of the tunnels – their configuration was altered from one day to the next on a random basis. One of the objects was an item imitating a green plant (O1) – see Fig. 2. The underlying assumption was that the tortoises would respond strongly to an object resembling their typical food. The second constant object was a yellow sphere, 10 cm in diameter (O2) – see Fig. 2. The third object (O3N) presented to the tortoises varied from one study session to the next over the course of the experiment. The variable objects were of different shapes and colours, yet did not diverge greatly from the constant objects in these respects – they were 5-10 cm wide and maximum of 10 cm high.

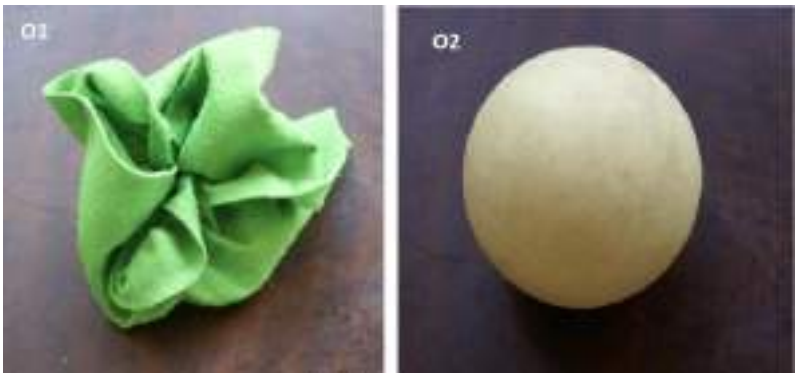


Fig. 2. Constant objects/stimuli used in the study.

The variable objects were the following (in the order in which they were presented): nail clippers, a cardboard matchbox, a car model, a pink nail polish, a small furry toy, a claret-coloured candle, a roll of sticky tape, a white porcelain glass, a measure tape, an empty grey toilet paper roll, a white mug, a white plug-in time switch, a “pepsi” glass, a small elephant figure, a pill box, a dark-blue and white light-bulb box, a grey furry toy rat, a roll of

white toilet paper, a white nailbrush, a small green plant in a pot, a pink highlighter, a bright-green cardboard box, a small jar filled with coffee, a 0.5 litre water bottle, a paper ball, a beige mug, a pill box, a blue pen, a small white plastic bowl, and an orange watering can cork.

*2.3 Procedure*

The duration of the study was 40 days divided into cycles of 5 days of study and 2 days of interlude. The tortoises were always studied in the same order and at the same time of day. They were fed green vegetables 5 times a week after the end of each study session. The observations were carried out during the day, in the morning, which coincided with the peak of tortoises' circadian activity in their natural habitat.

Each of the tortoises was taken out of their pens, transported to the research apparatus, placed under the dome (in a randomly selected direction), from where it could look a the objects presented through the openings in the dome. Measurements were taken of the time and frequency with which it stuck its head out in the direction of particular objects. The length of each observation session was 50 minutes for each of the tortoises. After the session, a given tortoise was put back in the pen, and the experimental space was rinsed with water.

A video camera was fixed above the experimental space to record the behaviour of the animals.

## 3. Results

Given the preliminary, exploratory nature of the study, the statistical procedures which were employed provide no accurate basis for testing directional hypotheses (see also Valentine, Aloe and Lau, 2015). However, in order to better illustrate the preliminary results obtained, Repeated Measures ANOVA (within subject design) was used for each of the individuals separately. Three dependent measurements (behavioural measures embedded in the stimulus object) were applied in the analysis with respect to each tortoise in the study, with each measurement session constituting a separate data record. The descriptive statistical data is presented in Table 1; the ANOVA results are shown in Table 2.

Table 1. Descriptive statistics of the measures taken in the study. Occurrence - total number of staring responses; Duration - total time spent on staring responses.

| Tortoise #1 | | | | | |
|---|---|---|---|---|---|
| Attribute | Min | Max | Average | Std-dev | Std-dev/avg |
| O1-occurrence | 0 | 39 | 12.4 | 9.95 | 0.80 |
| O2-occurrence | 0 | 12 | 3.83 | 3.68 | 0.96 |
| O3-occurrence | 0 | 29 | 7.43 | 6.54 | 0.88 |
| O1-duration | 0 | 1200 | 355.67 | 332.75 | 0.94 |
| O2-duration | 0 | 502 | 85.53 | 121.94 | 1.43 |
| O3-duration | 0 | 443 | 152.1 | 128.18 | 0.84 |

| | | | | | |
|---|---|---|---|---|---|
| Tortoise #2 | | | | | |
| O1-occurrence | 0 | 102 | 26.46 | 30.22 | 1.14 |
| O2-occurrence | 0 | 65 | 16.14 | 20.64 | 1.28 |
| O3-occurrence | 0 | 101 | 20.34 | 24.41 | 1.20 |
| O1-duration | 0 | 2240 | 308.31 | 464.30 | 1.51 |
| O2-duration | 0 | 575 | 109.57 | 161.27 | 1.47 |
| O3-duration | 0 | 1035 | 220.34 | 299.14 | 1.36 |
| Tortoise #3 | | | | | |
| O1-occurrence | 0 | 19 | 6.07 | 5.08 | 0.88 |
| O2-occurrence | 1 | 18 | 4.63 | 4.00 | 0.86 |
| O3-occurrence | 0 | 13 | 5.67 | 3.34 | 0.59 |
| O1-duration | 0 | 96 | 19.26 | 24.64 | 1.28 |
| O2-duration | 1 | 54 | 10.03 | 11.46 | 1.14 |
| O3-duration | 0 | 84 | 22.07 | 22.37 | 1.01 |

Table 2. Repeated Measures ANOVA across stimulus objects of the variables taken in the study, for each subject independently.

| | Number of staring responses | Duration of staring |
|---|---|---|
| Tortoise #1 | $F(2,89)=12.46$, $p<0.001$ | $F(2,89)=10.86$, $p<0.001$ |
| Tortoise #2 | n.s. | n.s. |
| Tortoise #3 | n.s. | $F(2,89)=2.79$, $p=0.069$ |

## 4. Discussion

The results obtained indicate the presence of a constant pattern in all three individuals studied. The sphere (O2) elicited the smallest number of responses involving looking intently at the object, while the plant-like (O1) and the variable objects (O3N) elicited such a response to a far greater degree. In the case of all individuals, there was high variability in measurements of specific responses in the subsequent measurement sessions. Nevertheless, the following conclusions may be drawn on the basis of the above-described preliminary study:

- the tortoises show an exploratory response which varies due to the object being studied,

- the stimulus which imitates a biologically significant object elicited more and longer-lasting responses in tortoises which involved the animals' looking intently at the object,

- the variable stimuli elicited more and longer-lasting responses in tortoises which involved the animals' looking intently at the object, as compared with the responses to the constant biologically indifferent stimulus.

It may be suggested, therefore, that land tortoises from the Mediterranean Basin (*Testudo hermanni*) and Central-Asian tortoises (*Agrionemys horsfieldii*) may constitute interesting study subjects in comparative psychology, in studies aiming to examine the evolution of

cognitive processes in amniotes.

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| Konceptualizacja | KM |
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