



Laidlaw Undergraduate Program

RESEARCH REPORT

Summer 2023

**Do *Drosophila melanogaster* engage in play-like
behavior?**

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Public report

Preface and acknowledgements

I would like to express my deepest gratitude to Lord Laidlaw and the Laidlaw foundation for making this experience possible. This endeavor would not have been possible without my supervisor Dr. Matthias Durrieu who allowed me the freedom to lead my research project while advising me with great feedback. Many thanks to Professor Pavan Ramdya who was a guiding light throughout my project. Finally, special thanks to everyone at the Neuroengineering Laboratory for their support and to Mrs Dan-Thi Nguyen for her continuous help during this extraordinary endeavor.

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Introduction

There is a long history of scholars examining play through an evolutionary lens, beginning with Darwin (1859) and extending to modern times (Bateson, 2005, Pellegrini, 2011; Pellegrini & Bjorklund, 2004; Pellegrini, Dupuis, & Smith, 2007; Power, 2000). However, Today play is rarely mentioned in conjunction with invertebrates, be they barnacles, earthworms, spiders, bees or flies. (Aldis, 1975; Bekoff & Byers, 1981, 1998; Fagen, 1981)

“Even insects play together, as has been described by that excellent observer, P. Huber, who saw ants chasing and pretending to bite each other, like so many puppies”¹

Nonetheless, whether fruit flies interact with inanimate objects as a form of play has never been systematically examined. Play behaviour can be generally separated into three major categories: social, locomotor and object play, although multiple categories may occur simultaneously (e.g. social object play). Social play comprises playful interactions between animals, usually between juvenile conspecifics, such as play fighting². Locomotor play involves intense and sustained body movements, such as running and jumping, without any apparent need to do so (e.g. Miller & Byers, 1991). Object play relates to inanimate objects (e.g. Heinrich & Smolker, 1998). Previous explorations of play behaviour by insects include anecdotal evidence for social play in ants³ and a study in young wasps⁴, both on behaviours analogous to play fighting. One must also cite a study that explores ball rolling in bumble bees⁵ and constitutes a great depiction of this paradigm.

1. Darwin, 1877

2. Pellis & Pellis, 2017

3. *Formica rufa*, Huber, 1820

4. *Polistes dominulus*, Dapporto et al., 2006

5. Hiruni Samadi Galpayage Dona, Cwyn Solvi, Amelia Kowalewska, Kaarle Mäkelä, HaDi MaBouDi, Lars Chittka, 2022

Why *Drosophila melanogaster*?

The fruit fly *Drosophila melanogaster* is a versatile model organism that has been used in biomedical research for over a century to study a broad range of phenomena. The general principles of gene transmission, linkage, sex determination, genetic interactions; molecular, biochemical and developmental genetics, chromosomal aberrations, penetrance and expressivity, and evolutionary change may all be admirably demonstrated by using the fruit fly.



Figure 1. *Drosophila melanogaster*

Additionally, during the works of my supervisor Dr Durrieu on object learning in *Drosophila*, he observed that the flies would often roll balls for no apparent benefit. The question of play then arises, do *Drosophila melanogaster* have the potential to show a play-like behavior that is ‘pleasurable’?

Experimental limitations

Here we show that *Drosophila melanogaster* demonstrate glass-beads rolling behavior that could be attributed to play. We found that ball rolling (1) did not contribute to immediate survival strategies, (2) was intrinsically rewarding, (3) differed from functional behaviour in form, and (5) was initiated under stress-free conditions. In order to be defined as animal play⁶, the fourth criterion must be fulfilled (4) repeated but not stereotyped behavior. Indeed, Pellis (1993) makes the point that play is distinguished from nonplay or the merely

6. "Defining Play: Can We Stop Playing Around?", *The Genesis of Animal Play: Testing the Limits*, Gordon M. Burghardt, 2005.

“play- like” on the basis of the amount of time devoted to the putative play behavior as well as the length of individual play bouts (Byers, 1999a). This specific criterion won't be fully satisfied as the available experimental tools, specifically the recording devices, did not allow fly tracking. It is therefore paramount to note that our experiments will allow us to determine whether fruit flies exhibit a preference for ball rolling without explicitly defining such behavior as object play.

Methods

Ethical note

No licences or permits were required to run these experiments. There is no legislation protecting fruit flies or official guidelines for looking after *Drosophila Melanogaster* in research.

Housing Conditions and Animal Care

Experiments were conducted inside the laboratory. Lab flies are kept in bottles containing a jelly-like food that is typically made from a mix of water, cornmeal, yeast, soy flour, malt extract, corn syrup, and agar. The bottles are closed by cotton wool to prevent the flies escaping and to keep mites and other nasties out. *Drosophila* cultures are kept in room temperature where the temperature does not range below 20 °C or above 25 °C. *Drosophila* are maintained in two different light cycles to facilitate the work of the doctors and engineers; the first cycle is from 6 am to 6 pm and the second cycle is from 6 pm to 6 am. Our study flies were kept according to the first light cycle.

Their breeding ratio is 1:3 (male: female). The male and the female are often differentiated (under the microscope) based on their size, markings on their abdomen and presence of sex combs following anesthetization with CO₂. However the flies were anesthetized using

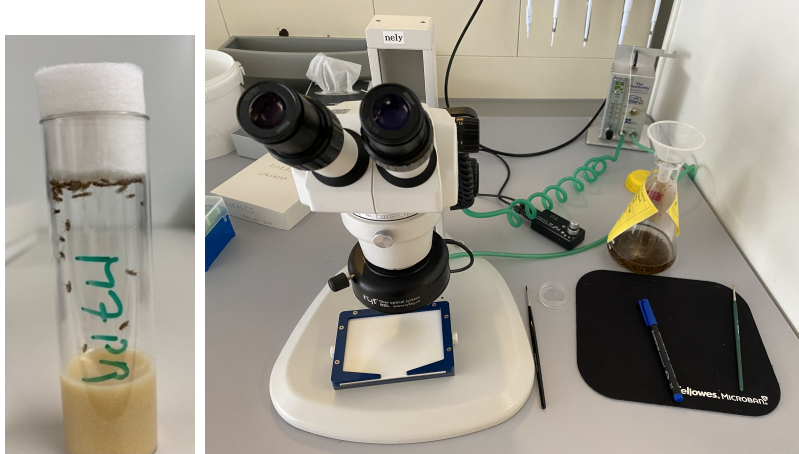


Figure 2. Lab flies

cold temperatures prior to the experiments to avoid the side effects of CO₂ anesthesia as it has shown effects on *Drosophila*'s physiology, stress tolerance, behavior, and fitness.⁷

Experimental setup

An arena was prototyped then built for this experiment using a laser cutter. The arena constitutes a living space for the flies during 24 hours with 5 different rooms. A playroom with movable beads, one with immovable beads, an empty one and a food room. The fruit flies were initially placed in the circular middle room at the beginning of each experiment. The height of the arena is 4 mm and each compartment (room) is 40x40 mm.

7. Shen, J, Yang, P, Zhu, X, Gu, Y, Huang, J, Li, M. CO₂ anesthesia on *Drosophila* survival in aging research. Arch. Insect Biochem. Physiol. 2020; 103:e21639. <https://doi.org/10.1002/arch.21639>

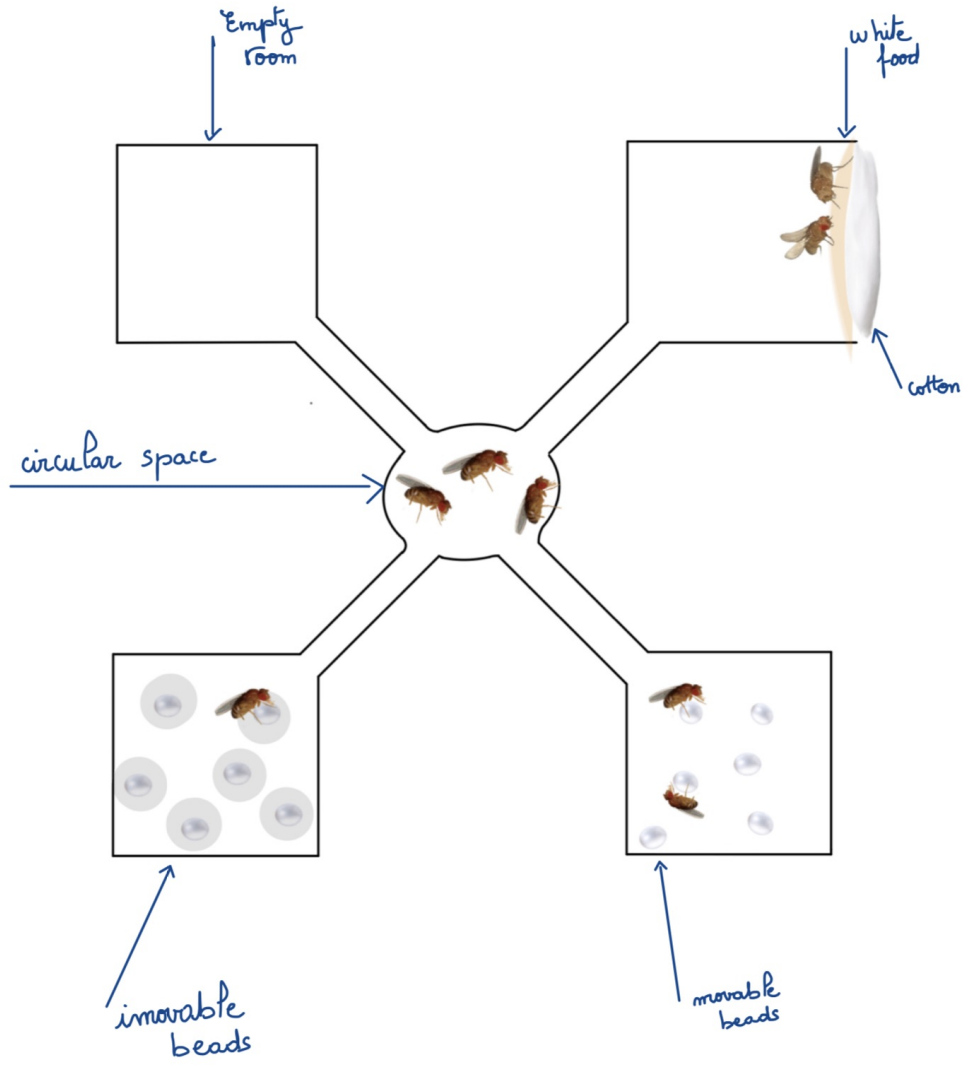


Figure 3. Fly arena

Recording devices

Logitech c270 webcams were used for the experiments, one camera for each arena with a frequency of an image per 12 seconds.

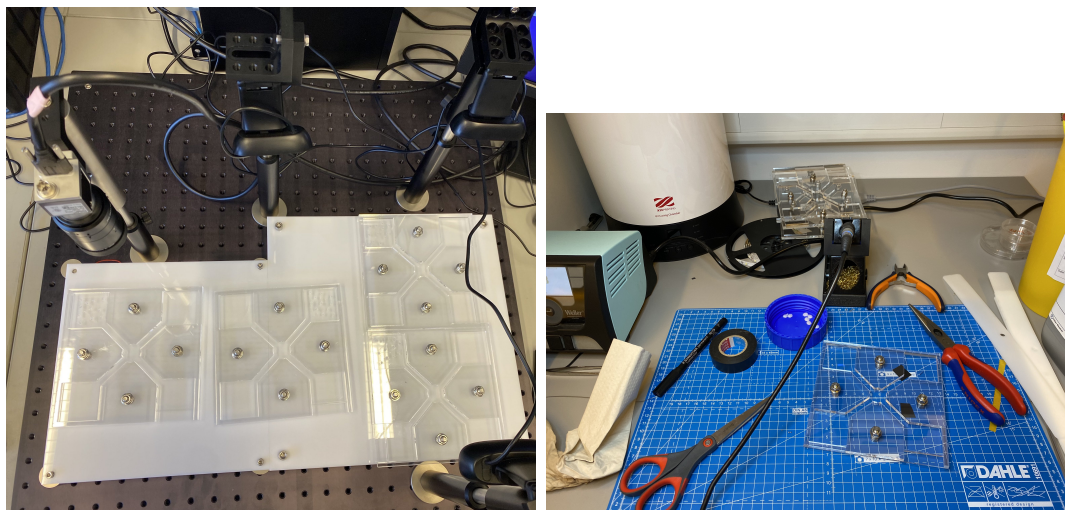


Figure 4. Building the setup

Experiment : Do fruit flies Engage in Play-like Object Manipulation?

Study animals

Female PR fruit flies (N=90) from the Neuroengineering lab were chosen for the experiment after observing that they were in good health (wings intact, size of the flies). Ages of fruit flies ranged from 1 to 9 days.

Objects

24 glass beads (1mm diameter) were used in each arena, 12 were glued using wax, and 12 others were distributed in the moveable beads playroom.

Video Analysis

Videos were analysed for the following information: number of flies in every room (including the central one) in each frame (5 frames per second). The images were analyzed

using python libraries: OpenCv, NumPy and panda. The second step consisted of training a model capable of detecting flies in the frames. In order to accomplish fly tracking , two deep learning platforms were used, robotflow and SLEAP. They both achieved a reliable model with a good prediction rate. It's important to note that the number of flies in the corridor was not taken into consideration in the video analysis.

Data Analysis

The 24 hours recording of the flies allowed us to plot the following graphs. In order to achieve this, we framed the pictures into the 5 different rooms. The first day of videotaping starts at 4 pm and ends at 9 pm. As we can see in this first graphic, the first 30 minutes exhibit exploratory behavior from the fruit flies. Indeed they spend some time in what appears to be specific exploration of their new environment. Specific exploration is an information extraction behavior or learning whether, for example, an object is prey, predator, or nesting material. Together exploratory and investigatory behavior reflect the presence of curiosity. Diverisive exploration typically follows specific exploration and is devoted to finding out what can be done with the object. This is what is traditionally called object play. Such diverisive exploration or play can involve manipulating, pushing, hitting, and over- turning. Exploration consists then of gathering information about the environment; whereas play with an object is finding out what can be done with the object.⁸

Following specific exploration, fruit flies seem to display a high preference towards the food room and a decreasing one for the other rooms. Such behavior can be associated with low energy at the end of their diurnal activity, or to put it more simply, flies are getting ready to sleep (Figure 5).

The second day of videotaping starts at 6:30 am and finishes at 4 pm. Entries into the areas with mobile and immobile balls were recorded. An entry was defined as a fly crossing the barrier from the clear path into an object area. Here we can observe an increasing preference for the mobile balls room followed by the immobile room.(Figure 6)

8. C. Hutt,1966; Renner, 1998

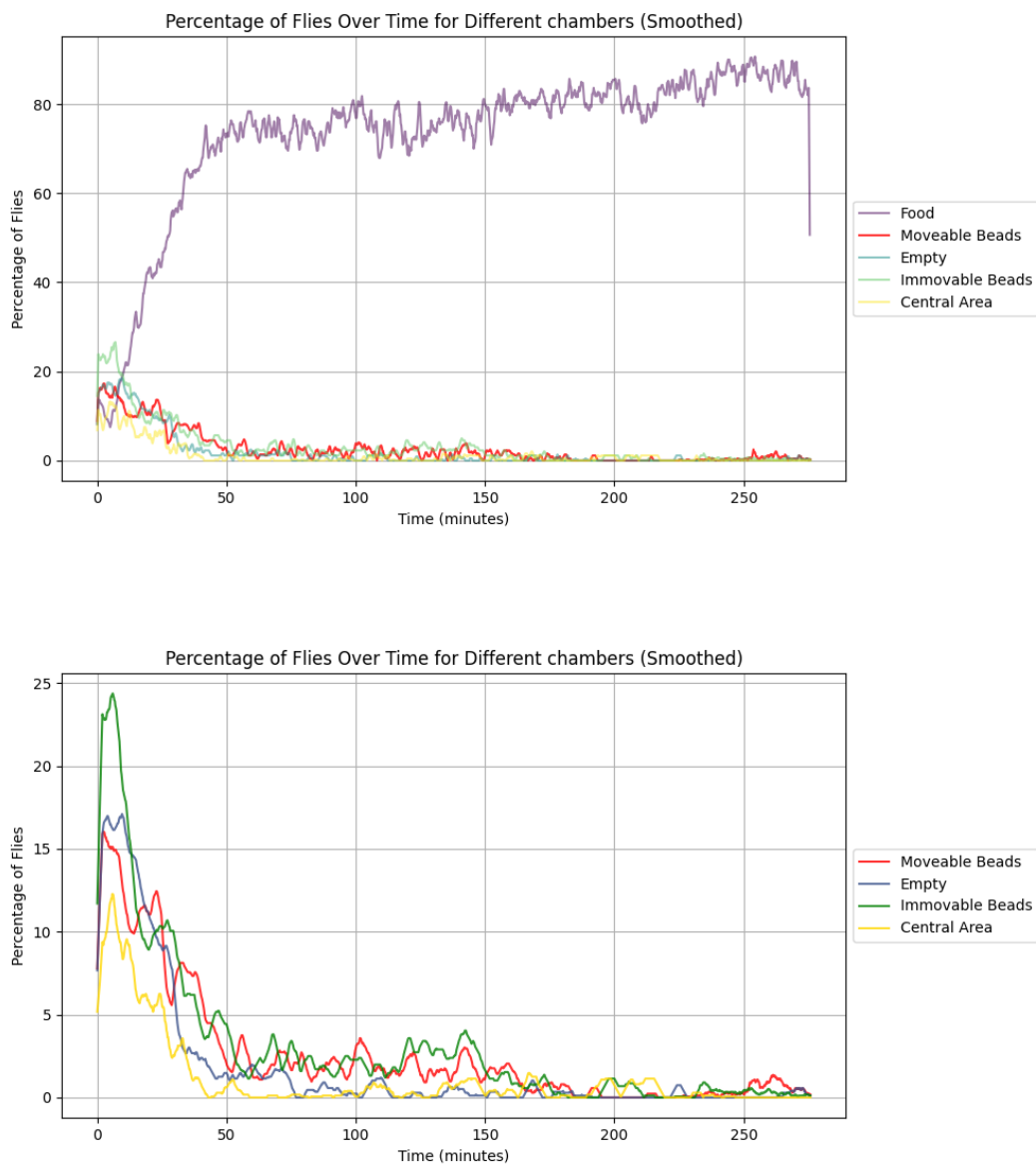


Figure 5. Fly activity during the first day

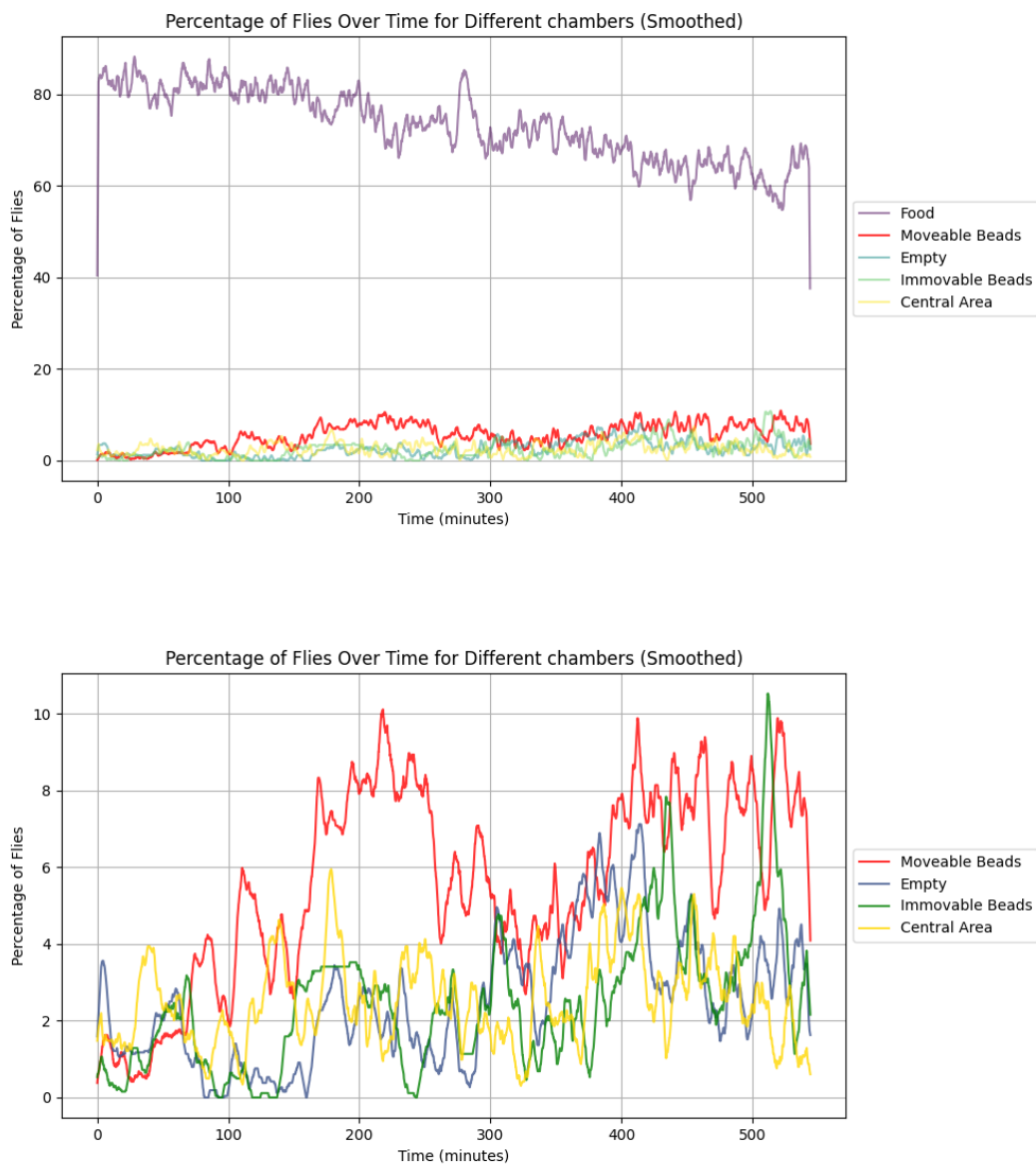


Figure 6. Fly activity during the second day

Behaviours recorded

Object interactions were actions where *Drosophila melanogaster* touched a ball. Ball rolling was defined as when an individual fly was on top of a ball and, while maintaining contact with the ball, rotated it along the floor. Such interactions were recorded in the following videos:



Figure 7. If you wanna see flies rolling two balls from the play room to the food room!



Figure 8. How about a close up to some...ball rolling!



Figure 9. Wow our flies are early birds!

In the last video, we can observe a change of activity at dawn. The flies spend the night in the food room then exhibit a sudden change in behavior expressed by many *va-et-vient* in the movable and immobile rooms, specifically the movable playroom.

Conclusion and Future directions

This work should help establish the grounds for more research on the ways in which play behavior might benefit early brain development.

Although by definition play should confer no clear, immediate benefit on the animal, species typical play should nevertheless be explicable in terms of survival value and reproductive success. This assumes (Lewontin 1978) that the behaviour is not merely of neutral selective value, resulting indirectly from some other adaptive change, or from genetic drift. In species where play is a significant part of the behavioural repertoire, the benefits should outweigh these costs. By definition, the benefits are not clear and immediate, although the costs are. It is most likely that there are benefits, but primarily delayed ones. The implications of this in life-history terms have been worked out by Fagen (1977). Depending on the ratio of delayed benefits to immediate costs, one would expect play (or play of a particular type) to be characteristic of species with a prolonged period of protected immaturity (costs minimised) and a long lifespan (benefits maximised). There would be no point in playful behaviour toward the end of the lifespan. Is this assumption true? Part of the future work that could be relevant to our paradigm would explore whether age and sex influence play behavior in fruit flies.

Furthermore, playful recombination of motor patterns and responses into new sequences could, like rearrangements of genetic material, tend to increase the phenotypic variability of an animal's offspring⁹.

Spread and elaboration of a novel behavior that may have originated in play is described by many scientists¹⁰. These "invented" patterns were later observed in goal-directed

9. Fedigan ;1972,Feitelson and Ross ;1973

10. Menzel ;1972, Gwinner ;1966

activity. Thus, the study of animal play may show the contribution of play as behavioral variation to evolution in invertebrates. Most importantly, a deeper understanding of animal play would allow us to design and develop AI and micro-robots capable of developing novel behaviors through environmental experimentation, specifically play, as an aspect of behavioral ontogeny in which partially or totally mastered responses are varied and combined into new sequences.