

How to study cognition (behavior)

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Introduction

Cognitive science and behavior science are interdisciplinary science studies of the mind and its processes. It examines the nature, tasks, and functions of cognition. Cognitive scientists study intelligence and behavior, with a focus on how nervous systems represent, process, and transform information. To study cognition we will be using “cognitive tasks”. Cognitive tasks we will be using are: Symmetric task (y-maze), Discriminative task (PER) and Psychophysical task (Stroop test). Symmetric task is a task in which you have to choose between three options based on sensory cues. Unlike symmetric task, discriminative task is a task in which, based on sensory cues, the subjects must have a “go/no go” reaction. Two possible outcomes are as follows; scenario in which subjects do a “go” action, and the other - where subject do a “no go” action. Reaction time and accuracy are direct measures of the subject performance. The third type is psychophysical: the experimentalist offers stimuli to the subject and observe perception and behavior that those stimuli produce. In this project we wanted to find a way to study cognition/cognitive science with, in our case, bees (*Apis mellifera carnica*). To do so, we organized a couple of experiments. Symmetric task experiment is called “Y-maze experiment”. With this experiment we aim to address the question “Can bees count?”. With the PER (Proboscis extension reflex) we used discriminative cognitive task, in which we trained bees to a certain odor while giving them food. After a Pavlovian conditioning, we want to know if they will react to the odor without presence of the food. Finally we used the Stroop task as a psychophysical task in which we wanted to show the struggles that brain goes through when to conflictual information comes in.

Proboscis extension reflex

Introduction

Proboscis extension reflex or PER, is a well establish experiment based on a discriminative cognitive task. This experiment is very similar to Pavlovs dog experiment: In these experiments, Pavlov trained his dogs to associate a unconditioned cue (the bell of a ring) and a reward (food). After a few trials, the dogs start salivating just after the bell sound showing an association between the sound and the reward. The dopaminergic system in the brain (also called the reward system) is responsible of this association. In our experiment we will do the same conditioning except we will stimulate bees with odor and associate a reward. There is no dopaminergic system in the bee, but the reward is controlled by a specific neuron responsible of the proboscis reflex. A proboscis is a specialized tool in bees and other pollinating insects, made up of upper and lower jaw ,tongue and upper lip. In this experiments, we wanted to see can bees recognize, distinguish and memorize different odors.

To address this question, we used previously mentioned PER. Indeed, bees don't possess much receptors for sweet on their tongues, but they have multiple on their antennas. To stimulate their tongue to come out there should be food presence on their antennas. We used this reflex to deliver the reward in the pavlovian conditioning. We show that bees are able to recognize an odor and associate it to a reward. They are also able to generalize this learnt knowledge to others odors, except if we specifically train them not to do so. Finally they are able to memorize an odor for a duration of at least few hours even after a few trials, indicating how important odors are in their life.

Methods

Catching bees

Before you start experimenting on bees you first have to have bees. Buying bees is not efficient and too expensive for just doing experiment on worker bees, so catching them by yourself is a best and most fun way to approach the situation. Best is to use standard Falcon tubes with pierced plastic caps. Then slowly took bees from flowers surrounding the beehive and hide them from the sun.

Freezing the bees

To properly contain bees while doing the training procedure first make sure they are immobilize and not a threat to you or your surrounding. Best way to do that is to make bees go to hibernation state which they do during the cold winter months. To make them hibernate artificially put the Falcon tubes with bees inside on ice. To make sure bees start their hibernation state, hold them inside for 3-5 minutes on ice.

Securing bees in immobilized state

If wished to conduct such experiment on bees they need to be contained. To do so use duct tape and a tube (diameter ~20mm, in which bees are placed straight out of freezer and tape them in a position in which their head is the only thing sticking out. Probably the most efficient way to handle this is to use 3D printer to make a small holding point for bees' head.

Training the bees

In order to see if bees can distinguish odors, we first have to make them connect odors with something they will find rewarding, like food or sugar. This way, they'll extend the proboscis and give an observable variable to monitor the learning. We trained bees using PER: After you apply sugar or something other sweet to antenas, bees will automatically stretch their tongue out in search of food then you just reward them with something sweet. Now, after seen that PER works, add another component to the training, odors. Odor is applied by a syringe with filter paper inside of it sprayed or rubbed with certain plant or perfume. The procedure we used is simple: Odor is applied for 5 sec and in the last 2 sec (4.th and 5.th) we provide simultaneously sugar to antennas and to the tongue. Between testings there should be 10 minutes intervals to starve the bees.

Results

We train a total of ~10 bees to associate an odor with a reward. We used 3 groups to test 3 different odors: lemon, melissa or perfum. After a few trials, the bees reacted with an extension of their proboscis, waiting for the reward, which indicate that they associate the odor with the reward. From this result we can conclude that bees are able to detect odors. We then wanted to know if a bee trained on a particular odor, can respond to another odor (ie. if the task is specific to a specific odor, or if they just associated the presence of something smelly to a reward). After the initial training, we presented new odors to the bees. We observed that the trained bees extended their proboscis showing a generalisation of the task. We can deduce that bees initially learnt to associate any odor to the reward. This result shows that bees are generalize instinctively the task. Following this experiment, we

wondered if bees are able to recognize an odor and to be specific. To answer this question, we trained the bees with a reward for an odor (A) and no reward for another odor (B). We noticed that after a few trials the bees associate the reward only with the odor A. There was no proboscis extension for the odor B. These results clearly show that bees have the ability to distinguish different odors. Finally, we wanted to know to which extent the bees learn the behavioral task and which type of memory they were using. The classical boundary between short-term memory and long-term memory is ~12 hours. We tested one bee on their rewarding odor after a night (~12h) and it was still performing the task correctly showing that the task was memorized in the long-term memory.

Discussion

Because of limited time we hadn't had a chance to test a lot of bees' reaction to two or more different odors. Similarly, the task to access memory was only tested on a couple of bees, leading to a small statistical power.

During training one bee slipped from her holding capsule so we were forced to immobilize her again, so we froze her. After putting it back in the holding capsule we noticed it doesn't remember its previous training. We trained it again to a different odor and it worked just fine but we had suspected that temporary freezing had made it lose its memory.

If we would have to change any part of our experiment it would probably be the holding capsule for the bees. Like I mentioned we taped the bees onto a neutral cylinder and although it worked, it was quite slippery for the tape and not really efficient so we would highly advise making 3D printed holding capsules because they might be more expensive but they are nicer to conduct this experiment.

Conclusion

In conclusion of this experiment we have understanding that bees can indeed smell odor, distinguish different odors and remember a task for days even after a few trials. This experiment showed us how intelligent bees really are and how fast they can learn.

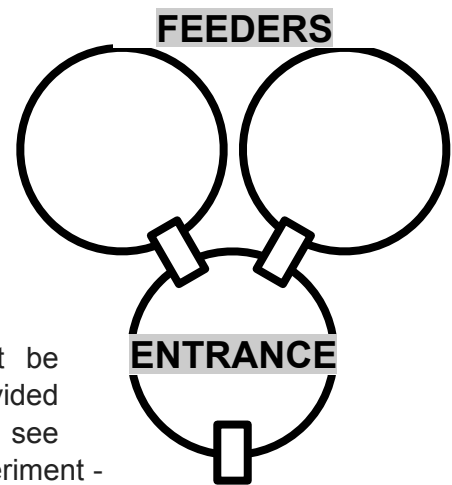
Y-MAZE EXPERIMENT

INTRODUCTION

Advanced behavioural patterns in bees have been well known to humans for thousands of years. Complex social structure encouraged bees to develop very elaborate thinking, logic and decision making skills throughout the evolution. But can they count? - that is the question we tried to answer with this experiment.

METHOD

Prior to any actual experimenting, a simple maze must be constructed in order to answer the question. The maze is divided into two main parts - the entrance, and two feeders. You can see on the scheme why this experiment is called the Y-maze Experiment - because of the maze's shape.



The three “rooms” are actually three cylinders connected to each other with short falcon tube tunnels. The entrance cylinder also has a hole through which bees can actually enter the maze from the outside. In the feeders, there are small containers which will eventually be filled with sugar solution. In order to be able to see into the cylinders, they are covered with glass. Since it would be very hard to sit by the maze and get the results in first person, it is advisable to set up a camera which will record the entrance where the results will be visible.

The used camera was a Raspberry Pi camera, connected to the homonymous computer which was connected to an external memory disk. The computer and the camera were previously programmed to capture an image every second because, for the sake of this experiment, high frame rate video is not a necessity.

Bees' ability to count will be tested by marking two entrances which lead to the feeders with different markings - different number of symbols, to be more specific. For example, three triangles will be put above one “door”, and two above the other. Food will be put in the feeder marked with three triangles, and it is the hypothesis that bees will memorise the number of symbols and know that the door with three triangles leads to food. One may think that bees might memorise which side to go to and therefore the experiment will not prove if they can actually count, and that is why the experiment will be controlled by switching the feeders together with their number of symbols, changing the symbols, their surface area, position etc. Also, sugar solution is used since it doesn't release any scent, so this will not affect the outcome of the experiment.

After all the practical work, the maze can be set up at the beehive. Since bees usually choose to go to well-known paths, they will have to be artificially introduced to the maze. To do so, a little bit of honey is put on a small piece of cardboard, and then in front of a random beehive where bees will start eating it. Then, the cardboard can be carefully removed and bees can be slowly put in front of the maze. After the same is done with multiple bees, they will be likely to memorise the location of the maze, and, eventually, “invite” other bees.

After some time, data can be gathered and analyzed. When the gathered video files are transferred to the computer, parts where there is movement need to be extracted in order to make data analysis quicker and more efficient. The computer must be programmed to recognize difference in pixelation between two images, only save those where there is something moving and track movement using lines. This way, moving patterns can be observed.

RESULTS

Unfortunately, because of bad weather conditions and short period we had available for this experiment, we were not able to answer the main question of it. Even though we could successfully track bees using the program, the hypothesis was not proven to be correct, nor incorrect due to lack of data.

DISCUSSION

Because of the unsuccessful outcome of the experiment, there aren't any major improvements we would suggest other than to plan the experiment according to the weather forecast and to, definitely, take your time and have a lot of patience.

It would also be a good idea to do the experiment in fully controlled conditions in a lab. If you collect bees prior to the experiment, the same ones will return to the maze all the time and therefore, the results would be much easier to analyse. When doing the experiment in the actual beehive, new bees come constantly and they might have to learn again every time. That makes data analysis very confusing because, as long as the bees aren't marked, we cannot know if the bee is new to the maze, or if it came back. That way, the results might not turn out to be very exact. On the other hand, working under controlled conditions might be a lot more expensive and technically challenging for the scientist. Bees would have to be put in a new beehive together with their queen, there would have to be water, food and also flowers for them to visit etc.

CONCLUSION

In conclusion, we cannot say that we proved our hypothesis, but we still believe it can be proven. Similar experiments have been done before with successful results ([Number-Based Visual Generalisation in the Honeybee](#) Gross HJ, Pahl M, Si A, Zhu H, Tautz J, et al. (2009) Number-Based Visual Generalisation in the Honeybee. PLOS ONE 4(1): e4263. <https://doi.org/10.1371/journal.pone.0004263>), so if you take your time to plan the experiment well and arm yourself with a lot of patience, with a little help of this report you can definitely prove that bees are so smart that they actually - can count.

STROOP TEST

Introduction

The Stroop test is a neuropsychological test, mostly used for measuring a subject's executive functioning, especially selective attention, cognitive flexibility and processing speed.

Stimuli

The test included three types of stimuli: incongruent stimulus and two control stimuli, namely congruent and neutral. In incongruent stimulus, the name of a colour is printed in an ink which conflicts the word's meaning. (Example: the word "red" written in blue ink.) The subject's brain gets confused by the contradiction, thus an extended reaction time is expected compared to the controls. In neutral stimulus, the word is either not a colour or a colour in a language which is unknown by the subject, thus there is no confusion caused. (Example: "XXX" written in yellow ink.) The reaction time is expected to be better than incongruent stimulus, but a bit slower than congruent stimulus. In congruent stimulus, the name of the colour matches the ink colour. (Example: the word "green" written in green ink) The reaction time is expected to be the quickest of all three, since the brain processes the meaning of the words quicker than the physical colour as a result of habitual reading.

Hypotheses

Our study aimed to assess the relationship between language proficiency and reaction time on the Stroop test. Two possible outcomes were predicted:

(A) Proficiency of spoken languages does not affect reaction time, thus we expect a constant reaction time with all incongruent stimuli, and a decreased reaction time with congruent/neutral stimuli.

(B) Proficiency of spoken languages does affect reaction time, producing a gradual decrease of reaction time with the decreasing proficiency of language in incongruent stimuli. Neutral/congruent stimuli is still expected to lead to a quicker reaction time, with congruent being the quickest.

Methodology

Participants

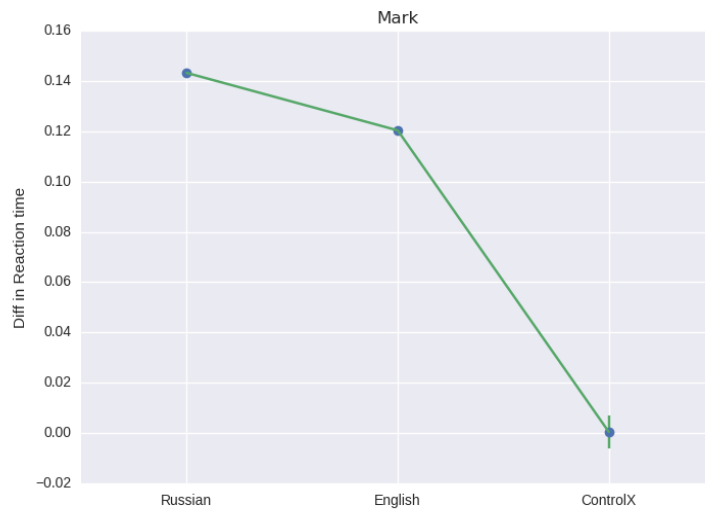
Seven people, aged 16 to 25 participated in the study. Every subject was tested individually.

Procedure

Subjects were assessed with a program written in Python, including all three types of stimuli. Firstly, participants were asked to give their spoken languages, their proficiency and the names of four predetermined colours in the given languages. Then, they had to give a response by pressing one of 4 keys on the keyboard with coloured pieces of paper attached to them. There were 100 incongruent stimuli, and 50 control given. The types of stimuli, languages, words and colours were randomised. The program measured participants' reaction time and error rate, and compiled a diagram with individual results at the end of the assessment.

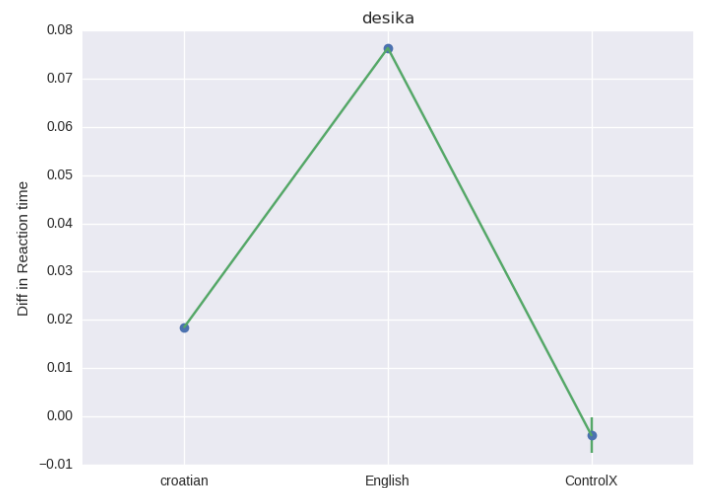
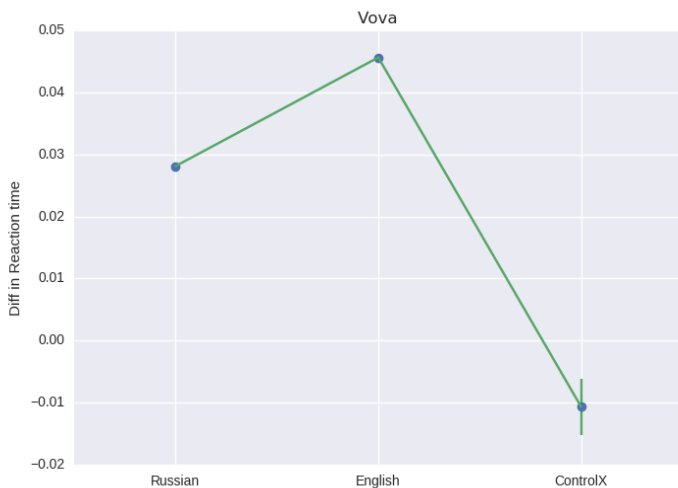
Results

(1)



The native language of this subject is Russian, and he is also very proficient in English. The diagram perfectly resembles the two language levels, and the reaction time to the control stimuli is low as expected. A gradual decrease in reaction time with language proficiency can be seen, proving outcome (B).

(2)



If we don't regard our subject's mother tongues as more proficient than English, more results prove outcome (B).

(3)

Results where reaction time for control stimuli is greater than other stimuli cannot be interpreted. Four subjects belong to this group.

Discussion

Since 4 out of 7 subjects were excluded (3), we cannot draw obvious conclusions. Only result (1) proved expected outcome (B) certainly, and no results proved outcome (A).

In results (2) regarding English as more proficient than our subject's mother tongues would explain the results. In this case, two more results suggest outcome (B).

We lack statistics to really determine the presence of either of the outcomes. Successful results imply expected outcome (B), which suggests that reaction time decreases gradually

in parallel with language proficiency, however further research is needed to verify this claim.

Our limitations included not having enough subjects and the lack of a neutral environment. Also to properly assess our hypotheses, more languages would have been needed per subject.

Conclusion

Because of our limitations, we cannot state anything certainly, however all successful results prove expected outcome (B), so we may conclude that reaction time decreases gradually in parallel with language proficiency.

CONCLUSION

In this study we investigate different types of cognitive tasks. Each task type is suitable for a particular type of cognitive question, and the experiment should be constructed carefully in order to reduce cognitive bias. We spend a lot of time conceiving the experiments to avoid such bias because you want to make sure the subjects (bees or students) are not cheating. Indeed, we were asking very specific questions so we wanted the subject to perform this very specific task (counting for example) and not use another trick to solve the task.

Even if we didn't have time to finish completely one experiment, we manage to learn and test important notion of cognitive neurosciences such as learning, reaction time and memory.