AI Model and Experimental Study of Drug Effects on Planarians' Regeneration

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Abstract—**Due to planarians' remarkable regenerative capabilities, they have been a popular biological model for researching the effects of drugs on tissue regeneration. These unique abilities provide insights into potential applications of regenerative medicine. Despite extensive research, machine-learning models have yet to be integrated into this field of study. This paper proposes the first artificial intelligence machine-learning models used to predict planarian morphology when exposed to different drugs and manipulations, leveraging datasets derived from 1716 experiments documented across 119 publications spanning from 1898 to 2018. Two machine learning models are explored in this paper: a random forest classifier model, and a deep learning neural network multi-output regression model. The initial results indicate that the random forest model achieved over 96% accuracy for predicting lethal, tail, and trunk morphologies, and 64%, 76%, and 80% accuracy for predicting pharynx, head, and eye morphologies. For the neural network regression model, the loss is at 0.18. Additionally, in order to understand drugs' effects on planarian morphology, learning, and memory, experimentation is conducted with different alcohol concentrations. This paper aims to facilitate the analysis of the effects of drugs on planarians using artificial intelligence technologies and to aid future experiments.**

Keywords—drug effects, planarian regeneration, artificial intelligence, machine learning, planarian morphology

I. Introduction

Planarians are free-living flatworms that can regenerate any missing tissues or body parts. These invertebrates have been experimented on by many researchers from diverse fields. Understanding planarians' regenerative mechanisms has been a central focus in various studies, as they attempt to reveal the complex processes that underlie this ability. Researchers underscore the importance of neoblasts, or pluripotent stem cells, in planarian regeneration. Planarians are also used for computational modeling [1], behavioral studies [2], toxicology, and pharmacology [3]. Planarian neurons have many morphological, electrophysiological, and pharmacological features that are similar to the vertebrate brain [4]. Planarians also have a central nervous system, excretory system, and intricate anatomical system, and they are bilaterally symmetrical like humans [2], making them a suitable biological model for investigating the effects of drugs on humans. Further, planarian neoblasts divide throughout a planarian's lifetime, and their offspring become integrated with the differentiated tissues and replace damaged cells [5]. This makes planarians an ideal experimental system to address current problems in stem cell research, such as the evolutionary conservation of pluripotency, dynamic organization of differentiation lineages, and understanding the mechanisms underlying stem cell homeostasis [6]. Developing machine learning models for predicting morphology can help future researchers analyze large datasets more efficiently and validate their experiments.

Planarians have commonly been used as a biological model to study regeneration in humans. Since their nervous system is very similar to humans because of their observable sensitivity to substances, they have also been used to test the effects of drugs or toxic chemical compounds on their regeneration. This experimentation can help us understand responses to drugs in more complex organisms like humans.

Drug effects on planaria have been reported by several groups. Among the earliest studies of the effects of drugs on planarians was by Pederson [7], dating back to 1958. His study investigated the effect of trimethylene melamine on the morphogenic activity during planarian regeneration. Further, subsequent research by [2] introduces the first evidence of cocaine and glutamate producing behavioral sensitization in planarians, displaying planarians' potential as an in vivo model for developing new methods to investigate how various drugs can affect planarians.

Following advancements in these experimental methods, Lobo et al. introduced the first algorithmic approach using computation principles to infer regulatory networks from experimental morphological outcomes [1]. This method produces network models that serve as testable hypotheses for explaining and understanding data patterns observed in planarian regeneration. Using computational approaches to analyze morphological results provides researchers with further experimental validation and expands the bounds of traditional empirical methodologies. This paradigm shift greatly advances the integration of computational sciences into regenerative medicine and developmental biology.

Building upon Lobo's approach, Ghosh updates recent applications of computational techniques within planarian research [8]. This research covers tools like InterMine and PlanMine [9] that incorporate automated pipelines to efficiently access, mine, and analyze planarian transcriptome sequence data. Additionally, tools such as ImagePlane facilitate the study of planarian morphology, allowing for more in-depth observations, especially when investigating RNAi-induced changes. These computational methodologies contribute to the understanding of molecular processes, immune responses, receptor identification, morphological analysis, and the complex regulatory networks coordinating regeneration and development in planarians.

The current experimental-reliant and computational methods used to investigate the resultant morphologies after exposure to drugs are labor-intensive, costly, and very time-consuming. There is an urgent need to develop a more effective approach utilizing artificial intelligence technology. Artificial intelligence provides a more efficient means of analyzing planarian regeneration morphologies after being exposed to different types of drugs and manipulations, ultimately advancing the field of regenerative and pharmacological research.

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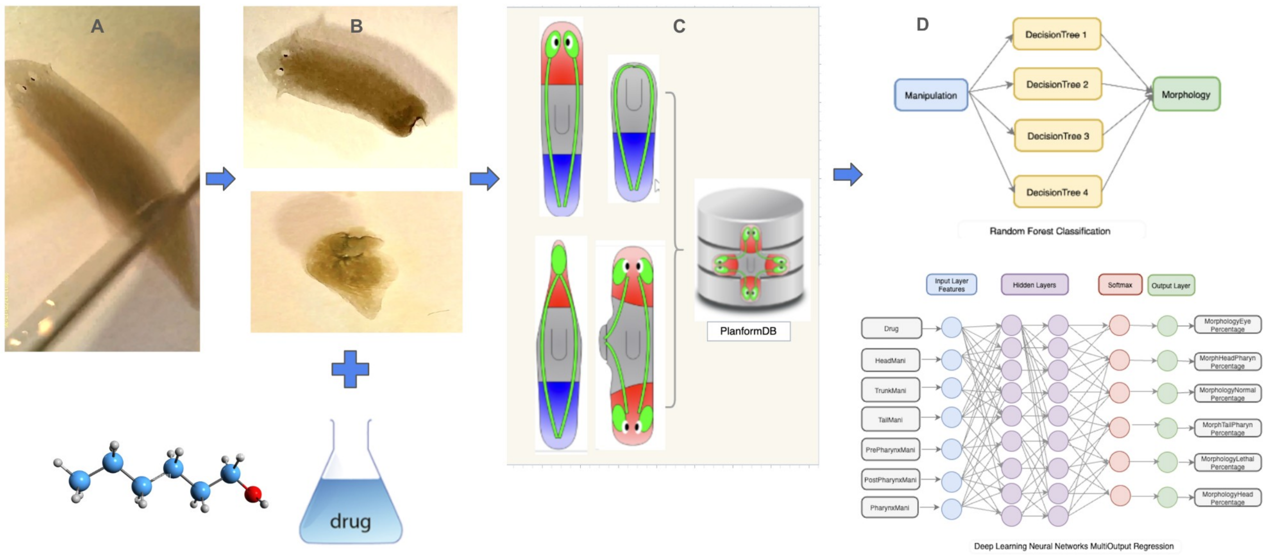

Figure 1 shows the diagram of the AI machine learning models for predicting drugs' effects on planarian regeneration morphology. It consists of four steps: A) manipulation by cutting the planarians B) treat the cut planarian pieces with drugs C) planarians morphed to different morphologies; import experimental results into the PlanformDB database [10] D) Leverage data from PlanformDB as datasets, use Random Forest Classifier Model to predict morphology classification and use Neural Network Multi-Output Regression Model to predict the percentages of each regeneration morphology

This paper proposes the first machine learning models that can predict the various resultant planarian morphologies when given the type of drug and manipulation the planarian is subjected to, developing a more efficient way to analyze larger datasets.

II. MACHINE LEARNING DATA SETS AND METHODS

A. Database

The data from PlanformDB [11], an experiment-phenotype database containing over 1716 experiments, 372 morphologies, 37 drugs and 284 manipulations, derived from 119 literature publications spanning from 1898 to 2018, served as a source for the datasets to develop and train the machine learning models in this study. Figure 2 displays the database schema of PlanformDB. PlanformDB contains experimental data for morphology after manipulation and treatment with drugs and RNAi.

This study focuses on the following alcohol compound drugs: heptanol ($C_7H_{16}O$), hexanol ($C_6H_{14}O$), octanol ($C_8H_{18}O$), and ethyl alcohol (C_2H_5OH), making up a total of 124 recorded experiments in PlanformDB, which are used as the dataset for the machine learning models.

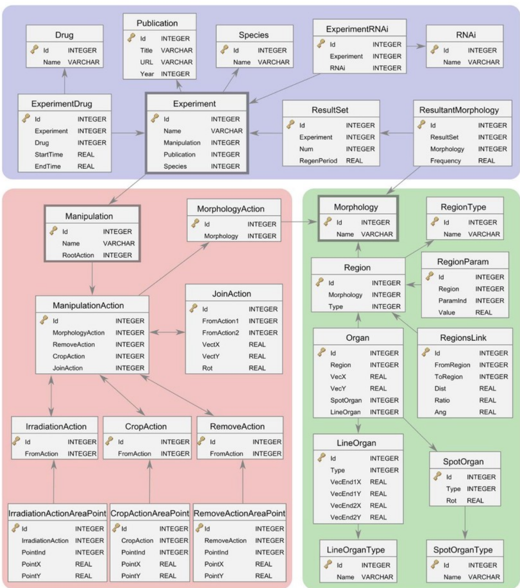

Figure 2: PlanformDB Schema [11]

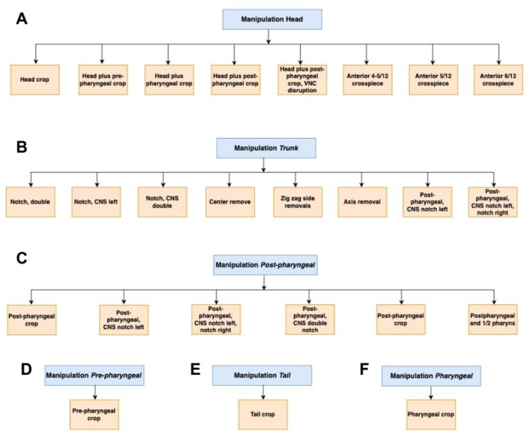

Figure 3: Feature Selection - manipulation categorization. A) head manipulations that are categorized as Manipulation Head B) trunk manipulations that are categorized as Manipulation Trunk C) post-pharyngeal manipulations that are categorized as Manipulation Post-pharyngeal D) pre-pharyngeal crop categorized as Manipulation Pre-pharyngeal E) tail crop categorized as Manipulation Tail F) pharyngeal crop categorized as Manipulation Pharyngeal

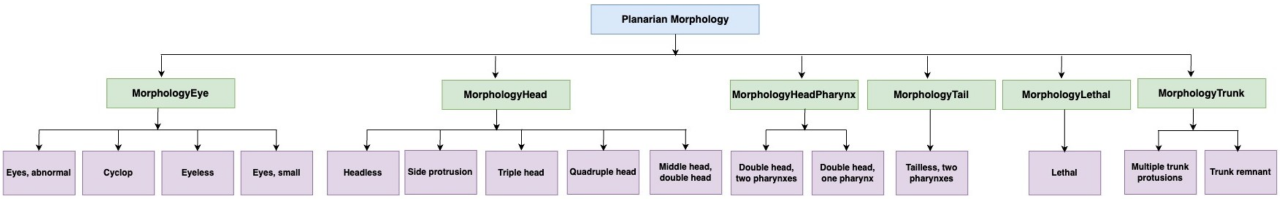

Figure 4: Target Selection - morphology categorization. Different planarian morphologies are categorized into MorphologyEye, MorphologyHead, MorphologyHeadPharynx, MorphologyTail, MorphologyLethal, and MorphologyTrunk based on the characteristics of the morphologies in the database

B. *Machine Learning Model Development*

1) Feature Selection and Categorization

In Figure 3, the manipulations in the database are categorized according to their characteristics. These categorized groups are used as features for the machine learning models.

2) Target Selection and Categorization

In Figure 4, the morphologies in the database are categorized according to their distinctive characteristics. For example, "MorphologyEye" indicates an eye-related issue observed post-regeneration. These categorized groups are used as targets for the machine learning models.

III. Machine Learning Models Results and Discussion

This paper evaluates two machine learning models: a random forest classifier model, and a deep learning neural network multi-output regression model.

A. *Random Forest Classifier Model*

Random Forest Classifier Model reduces overfitting by averaging multiple decision trees, increases generalization, and handles high dimensional data well. It is a fitting model for the purposes of classifying manipulations into resultant regeneration morphologies. The model uses 80% of the dataset as training data and 20% of the dataset as test data. 100 decision trees were used (n_estimators = 100) and random_state = 42. As shown in Figure 5, the model's accuracy for different morphologies ranges from 64% to 100%.

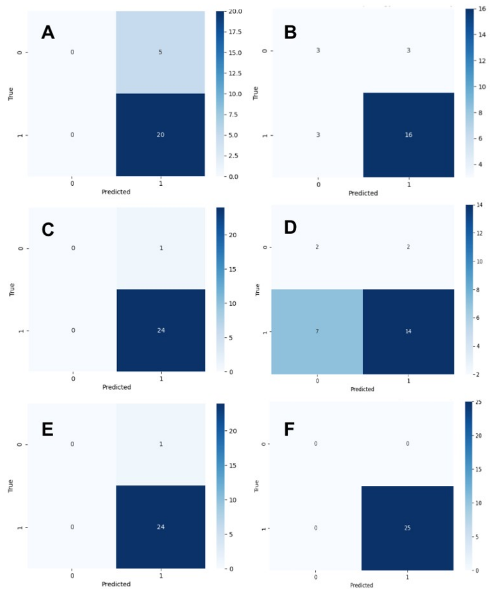

Figure 5: Confusion Matrices and accuracies for each morphology predicted by random forest classifier model. A) Prediction for MorphologyEye, accuracy 80%. B) Prediction for MorphologyHead, accuracy 76%. C) Prediction for MorphologyLethal, accuracy 96%. D) Prediction for MorphologyPharynx, accuracy 64%. E) Prediction for MorphologyTail, accuracy 96%. F) Prediction for MorphologyTrunk, accuracy 100%.

B. *Deep Learning Neural Network Multi-Output Regression Model*

The Neural Network Multi-Output Regression Model is designed to predict multiple targets simultaneously, making it well-suited for predicting percentages of regeneration morphologies. In this model, planarian manipulation and drugs are used as the input and the percentage of each resultant regeneration morphology is used as the output. The model uses 80% of the dataset to train the model and 20% of the dataset as test data. Table 2 is the dataset sample where the output of morphologies' percentage add up to 100%. The model uses random_state = 42 and hidden_size = 64. The softmax activation function is used to normalize the targets so the resulting morphology percentages sum up to 100%. For the training loop, num_epochs = 500. The model uses optimizer.zero_grad. Table 1 shows the model's performance.

Table 1: Neural Network Multi-Output Regression Model

Epoch	*Loss*
100	0.1936
200	0.1889
300	0.1882
400	0.1880
500	0.1878

Validation Loss	0.2589
Mean Squared Error (MSE)	0.0444
R^2 Score (Coefficient of Determination)	0.0609

Table 2: Neural Network Multi-Output Regression Model Data Set Sample: Morphology Percentages Sum to 100%

DrugName	*HeadMani*	*TrunkMani*	*TailMani*	*PrePharynx Mani*	*PostPharynx Mani*	*Pharynx Mani*	*MorphEye*	*MorphHead Pharyn*	*MorphTail Pharyn*	*MorphHead*	*Morph Lethal*	*Morph Normal*	*ExperimentName*
Heptanol	0	0	0	0	0	1	0	0.18	0.25	0	0	0.57	Nogi 2005 Fig. 10III, 3
Hexanol	0	0	0	0	0	1	0	0	0.2	0	0	0.8	Nogi 2005 Fig. 10III, 2
Heptanol	1	0	0	0	0	0	0	0	0.17	0	0	0.83	Nogi 2005 Fig. 10IB, 1
Heptanol	0	0	0	1	0	0	0	0	0.36	0.16	0	0.48	Nogi 2005 Fig. 10IB, 2
Heptanol	0	0	0	0	0	1	0	0.04	0.59	0.05	0	0.32	Nogi 2005 Fig. 10IB, 3
Heptanol	0	0	0	0	1	0	0	0	0.19	0	0	0.81	Nogi 2005 Fig. 10IB, 4
Heptanol	0	0	1	0	0	0	0	0	0	0	0	1.0	Nogi 2005 Fig. 10IB, 5
Octanol	1	0	0	0	0	0	0	0.05	0	0	0	0.95	Oviedo 2010 Fig. 1A, 1
Octanol	0	0	0	1	0	0	0	0.28	0	0	0	0.72	Oviedo 2010 Fig. 1A, 2
Octanol	0	0	0	0	0	1	0	0.5	0	0	0	0.5	Oviedo 2010 Fig. 1A, 3
Octanol	0	0	0	0	1	0	0	1.0	0	0	0	0	Oviedo 2010 Fig. 1A, 4
Octanol	0	0	1	0	0	0	0	0	0	0	0	1.0	Oviedo 2010 Fig. 1A, 5
Octanol	1	0	0	0	0	0	0	0.04	0	0	0	0.96	Oviedo 2010 Fig. 1B, 1
Octanol	1	0	0	0	0	0	0	0	0	0	0	1.0	Oviedo 2010 Fig. 1B, 2

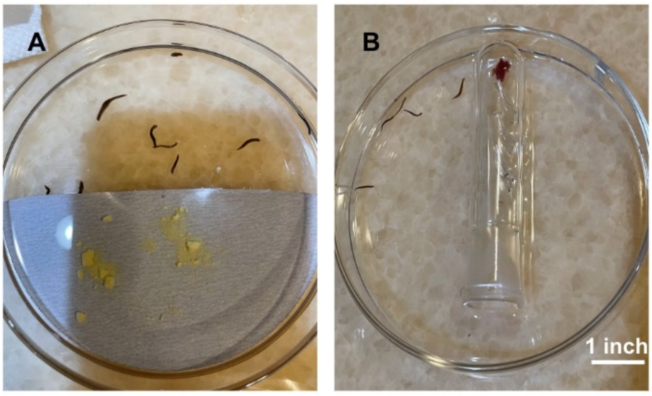

Figure 6: Two designs of settings to test drugs' effects on the learning and memory of planarians. A) egg yolk placed on rough surface as a reward B) beef liver put into a glass planarian trap

IV. Experimental Materials and Methods

In addition to creating machine learning models, experiments were conducted using varying concentrations of alcohol on planarians to confirm the resultant morphologies predicted by the models. The alcohol concentrations used during experimentation consisted of a control group of spring water, low dose of 1% alcohol (v/v), medium dose of 1.25% alcohol (v/v), and high dose of 1.5% alcohol (v/v). The initial results from the experimental methods showed that when the planarians were exposed to the low dose and medium dose of alcohol, they survived for more than 30 days. After exposure to the high dose for two days, one planarian lost its head and two lost their mobility. Four days after exposure, one of the planarians had a 0.025 cm large hole in its body, and all the planarians died after 11 days. These experimental results mirror the trends of the effects of alcohol on the planarians' regeneration morphology, further verifying the machine learning models. The results can be entered to the PlanformDB database as new dataset for the machine learning models.

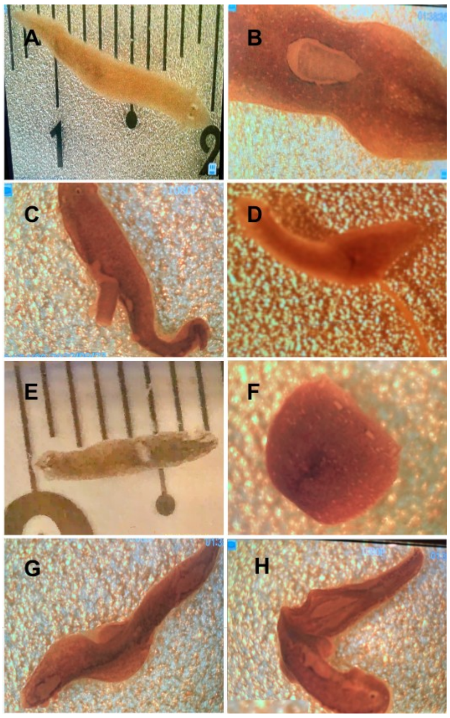

Figure 7: Physical effects after medium and high doses of alcohol treatment. A) normal untreated planarian B) a hole appeared in the middle of the body C) pharynx stuck out and hole in the body D) pharynx stuck out E) small hole appeared in the middle of the body F) withdrew into a small ball G) bumps appeared on the body. H) multiple holes in the body

A. Planarian Learning and Memory Test Setting

Figure 6 displays two designs of experimental settings used to study drugs' effects on the learning and memory of planarians. The design shown in Figure 6A has egg yolk placed on a rough surface to train the planarians to associate rough surfaces to food as a reward. Figure 6B has beef liver put into a glass planarian trap to train the planarians to enter the planarian trap to find the beef liver. After experimentation, Figure 6B was found to be a more effective method to train the planarians to find the food.

Table 3: Neural Network Multi-Output Regression Model Dataset sample: Output of morphology percentages add up to 100%

	Control Group	*Low Concentration 1% (v/v)*	*Medium Concentration 1.25% (v/v)*	*High Concentration 1.5% (v/v)*
Sample Size	10	10	10	10
Behavior	Normal	• Acted erratically • Survived for 3 months	• Acted erratically • Lost learning ability • Body twisted into different forms • Survived for 1 month	• Lost mobility • Lost learning ability • Body twisted into different forms • One day later, one lost head • Three days later, holes appeared in its body • Ten days later, they all died
Natural Regeneration	4 out of 10	2 out of 10	None	None

B. Planarian living environment

The planarians, specifically Girardia tigrina, were purchased from Carolina Biological. They were placed in room temperature spring water and kept in a dark environment due to their dislike for light. The spring water was changed everyday to maintain a clean living environment for the planarians. They were fed once a week with beef liver or egg yolk. Beef liver was preferred by planarians over the egg yolk.

C. Alcohol effects

Figure 7 displays the physical effects after exposure to high and medium doses of alcohol. Figure 7A is the control group of planarians, raised in spring water. Figure 7B shows a hole that appeared in the middle of the planarian's body. Figure 7C has a planarian with a protruding pharynx and a hole in its body. Figure 7D also shows a planarian with its pharynx sticking out. The planarian in Figure 7E has a small hole that appeared in the middle of its body. Figure 7F shows a planarian that withdrew into a small ball from exposure to alcohol. Figure 7G shows a planarian with an exploded body. Figure 7H has a planarian with multiple holes in its body. These all show possible resultant morphologies or the negative effects that drugs have on the planarians.

D. Alcohol concentration and effects on behavior and regeneration

Different alcohol concentrations are experimented with to investigate its effects on planarian's behavior and regeneration as shown in Table 3. The control group, low dose group, medium dose group, and high dose group all have sample sizes of ten planarians. The highest dose of alcohol showed the most significant negative effects on the planarians, as they lost their mobility, lost their learning ability, and their bodies twisted into different forms. Eventually all of the planarians died from the high dose of alcohol.

V. CONCLUSION

This paper is the first report using artificial intelligence to predict planarian morphology when exposed to different drugs and manipulations. Two types of machine learning models are investigated: a random forest classifier model, and a deep learning neural network regression model. Initial results indicate that the random forest model achieved over 96% accuracy for predicting lethal, tail, and trunk morphologies, and 64%, 76%, and 80% accuracy for predicting pharynx, head, and eye morphologies. The neural network regression model reported a minimal loss of 0.18. In the future, additional models will be implemented and more drugs other than alcohol will be explored and added to the dataset. Further optimization is in progress, involving model tuning and obtaining more data via experimentation for the dataset. Updates on this research will follow as more data is collected and analyzed.

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