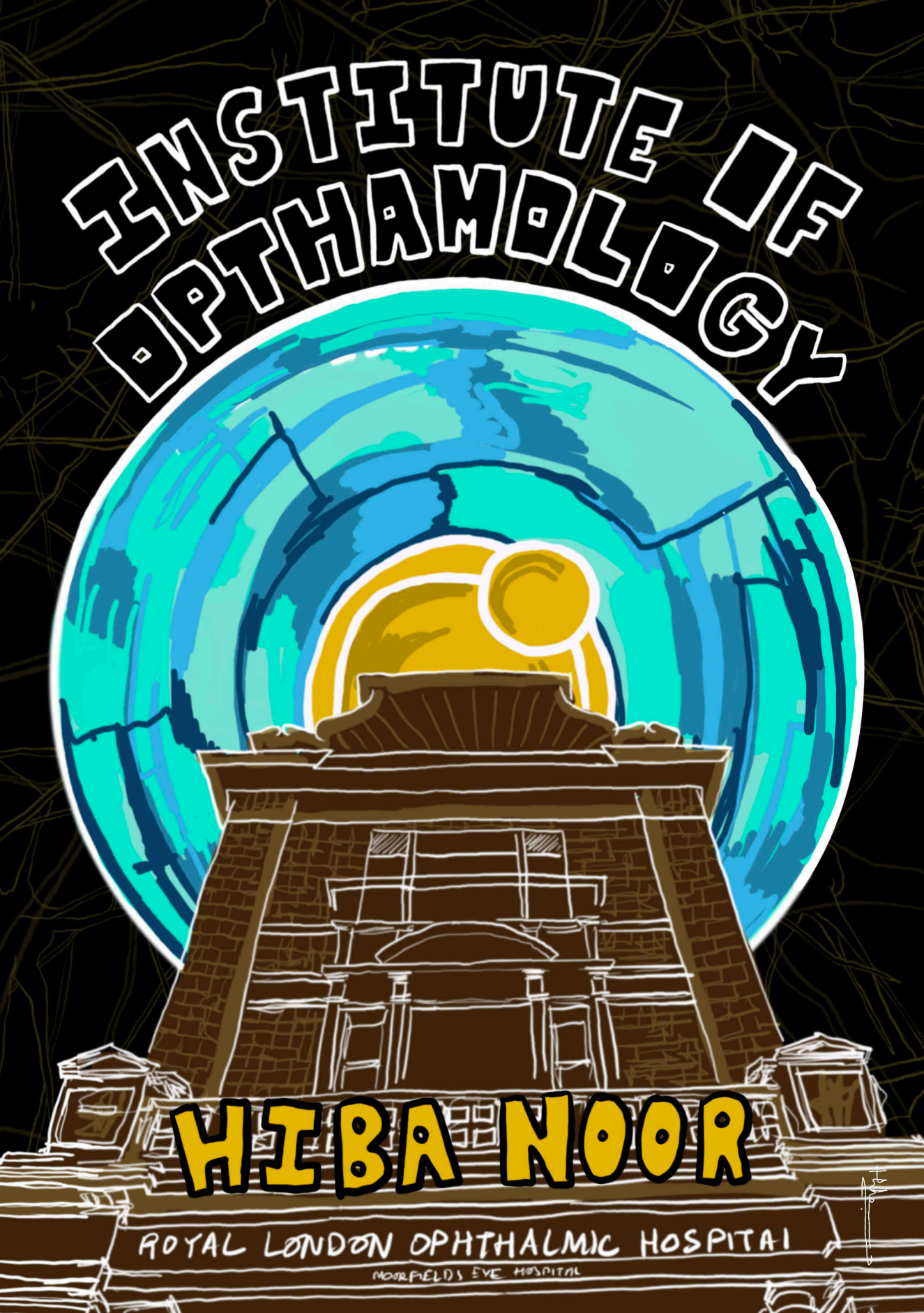
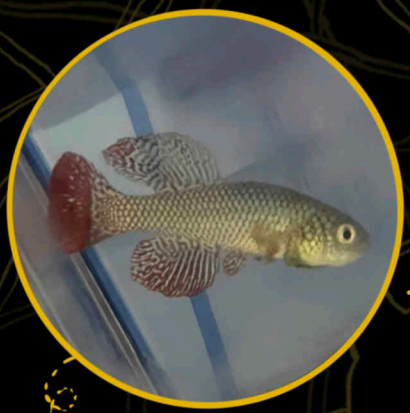

INSTITUTE IF OPTHAMOLOGY
HIBA NOOR
ROYAL LONDON OPHTHALMIC HOSPITAL
MOORFIELDS EYE HOSPITAL



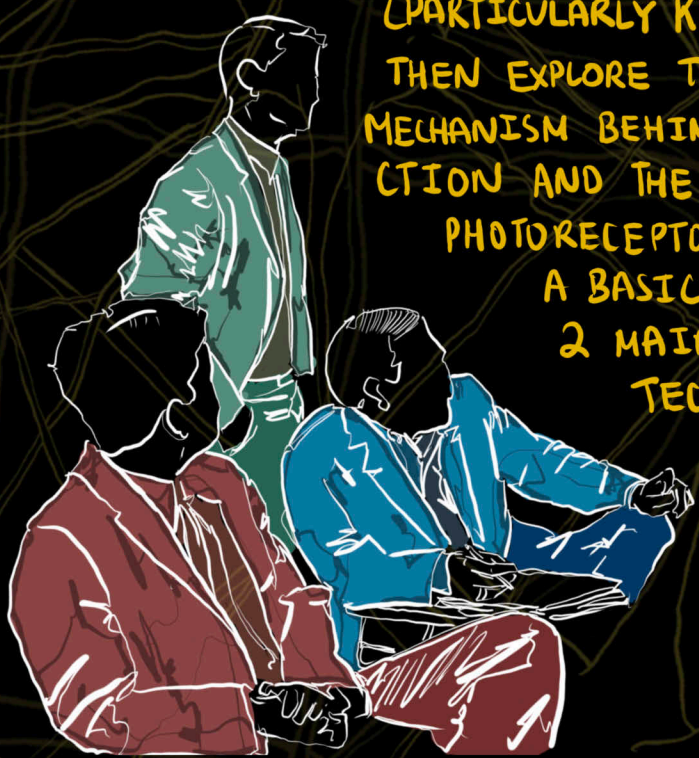
MY KILLIFISH PROJECT

HEY GUYS,

I TOOK PART IN A 8-WEEK INTERNSHIP AT THE MACDONALD LAB. I WORKED WITH DR NICOLE TO CHARACTERISE THE KILLIFISH RETINA, VERIFY ANTIBODIES AND EXAMINE OPTIMUM PROTOCOL.

THE AFRICAN TURQUOISE KILLIFISH IS A NOVEL SHORT-LIVED MODEL ORGANISM. ITS USED TO UNDERSTAND MORE ABOUT NEURODEGENERATIVE DISEASES.

IN THIS BOOKLET WE EXPLORE THE IMPORTANCE OF ANIMAL MODELS (PARTICULARLY KILLIFISH). WE WILL THEN EXPLORE THE UNDERLYING MECHANISM BEHIND VISUAL TRANSDUCTION AND THE SIGNIFICANCE OF PHOTORECEPTORS. FOLLOWED BY A BASIC INTRODUCTION TO 2 MAIN EXPERIMENTAL TECHNIQUES : IN-SITU HYBRIDISATION (HCR) AND IMMUNO-HISTOCHEMISTRY (IHC).



CONTENT


HIBA
NICOLE

ENGLAND IS AGEING

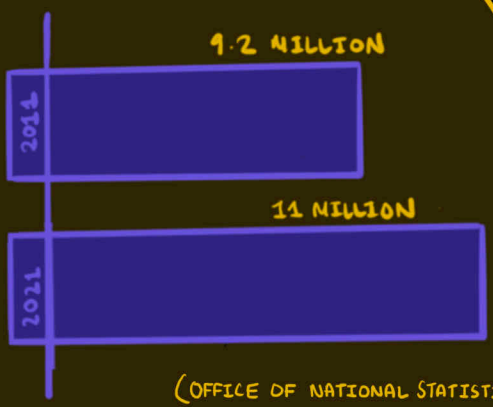
AGEING IS A SIGNIFICANT RISK FACTOR FOR DEGENERATION OF THE RETINA.

SOME OF THE MOLECULAR MECHANISMS WE SEE IN OTHER ORGANISMS UNDERLY HUMANS. HENCE ANIMAL MODELS ARE A VALUABLE SOURCE IN UNDERSTANDING THE BASES OF AGEING.

WE WILL LOOK AT THE RETINA AND MODELS OF RETINAL DISEASES - SHOWN TO BLIND THE ELDERLY POPULATION

THE EFFECT OF AN AGEING SOCIETY HAS LEAD TO A GLOBAL PUBLIC HEALTH CONCERN. THIS IS BECAUSE OF THE RETINAL DISEASES THAT DOWNSTREAM LEAD TO VISION LOSS.

AGEING POPULATION IN ENGLAND 65>



(OFFICE OF NATIONAL STATISTICS)

THE POPULATION OF ENGLAND HAS CONTINUED TO RISE SINCE 2011.

THE POPULATION OF ELDERLY HAS RISEN FROM 9.2 MILLION TO 11 MILLION.

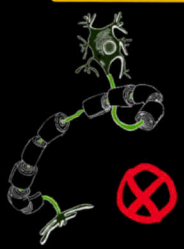
RETINAL DISEASES

A PROLONGED LIFE SPAN MEANS INCREASED PREVELANCE TO AGE-RELATED DISEASES...

AGE-RELATED DISEASES INCLUDE

NEURODEGENERATIVE DISEASES

ALZHEIMERS



- DAMAGED NEURONES
- AGGREGATION OF PROTEINS

RETINAL DISEASES

AGE-RELATED MACULAR DEGENERATION

- BLUR OF THE CENTRAL VISION DUE TO DAMAGED RETINA

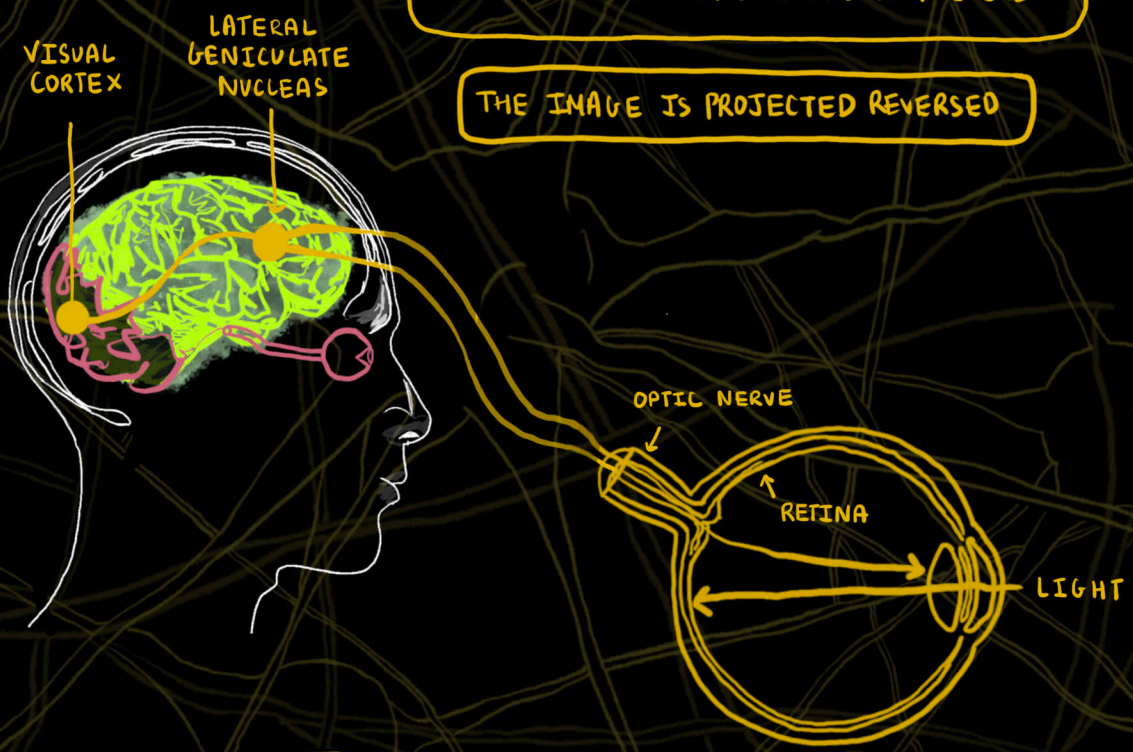
GLAUCOMA

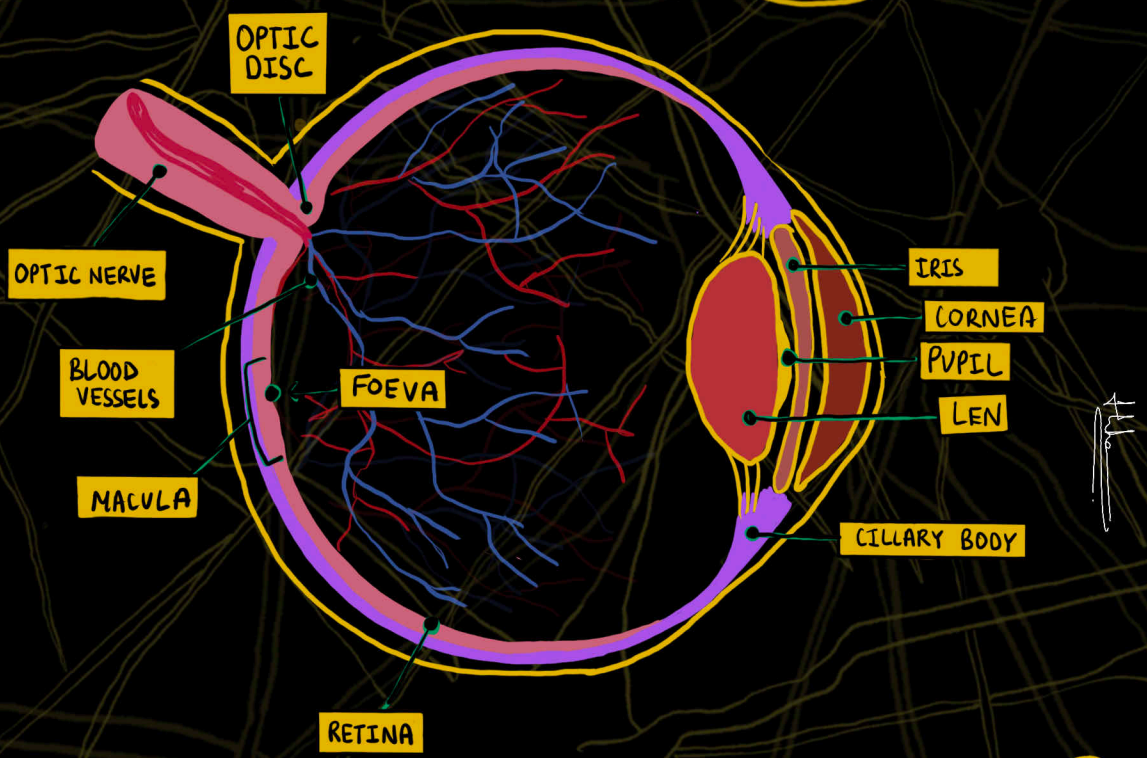
- DAMAGE TO OPTIC NERVES

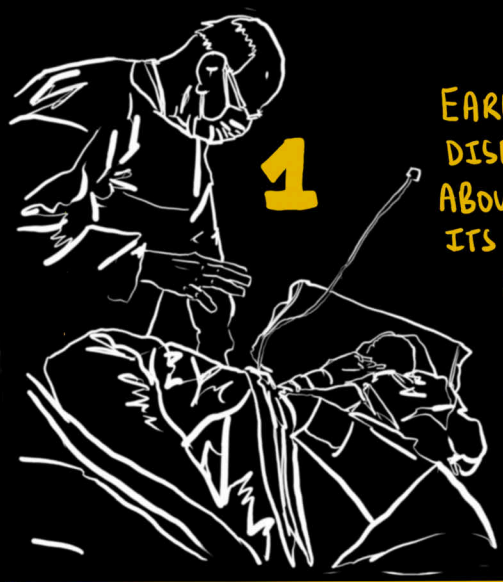
- THIS LEADS TO VISION LOSS

THE EYE IS AN ORGAN THAT TRANSLATES LIGHT ENERGY INTO CHEMICAL ENERGY WHICH IS THEN TRANSMITTED TO THE CNS

THE IMAGE IS PROJECTED REVERSED







1

EARLY DISCOVERY OF NOVEL EYE DISEASE. NOBODY KNOWS ANYTHING ABOUT THE DISEASE OR ITS UNDERLYING MECHANISM.

SO WHAT HAPPENS?

THE CLINICS OBSERVE THE DISEASE AND THE EFFECTS OF IT. RECORDING SYMPTOMS AND ANALYSING TRENDS IN DIAGNOSIS.

WHILE THIS HAPPENS STUDIES ARE BEING UNDERTAKEN TO UNDERSTAND MORE ABOUT THE DISEASE.

WE CALL THESE PRE-CLINICAL TRIALS.

2

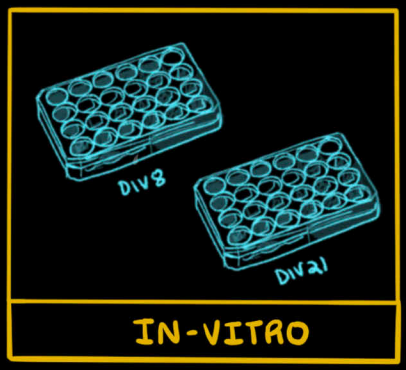
3

PRE-CLINICAL TRIALS ARE IMPORTANT BECAUSE EXPERIMENTS HELP INVESTIGATE CAUSE OF DISEASE.

SCIENTISTS LEARN MORE ABOUT THE BIOLOGICAL PATHWAY.

EXPERIMENTS ARE CARRIED IN-VITRO (OUTSIDE A LIVING ORGANISM) AND IN-VIVO (INSIDE A LIVING ORGANISM)

IN-VIVO



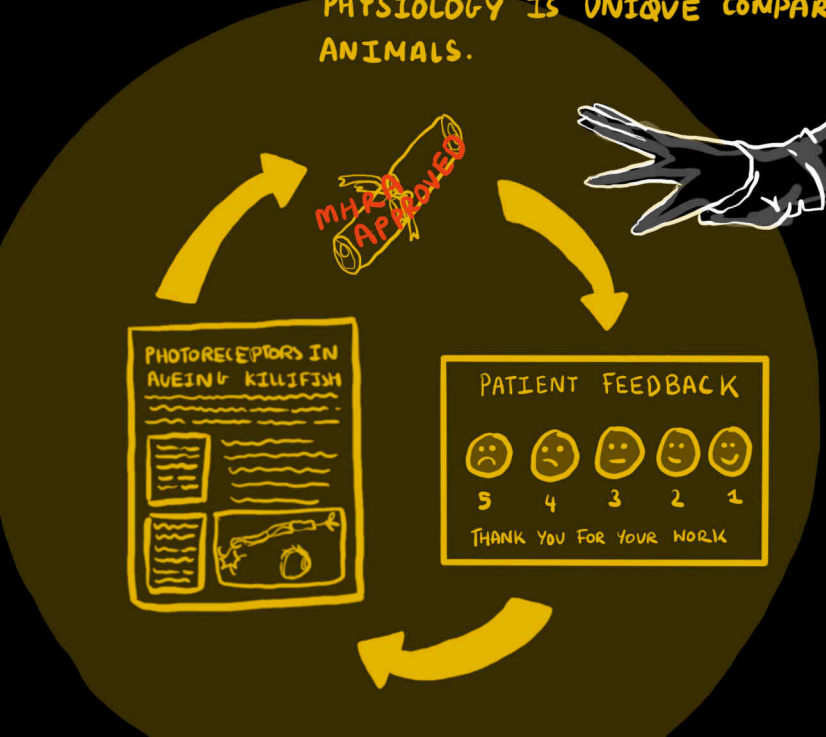
IN-VITRO

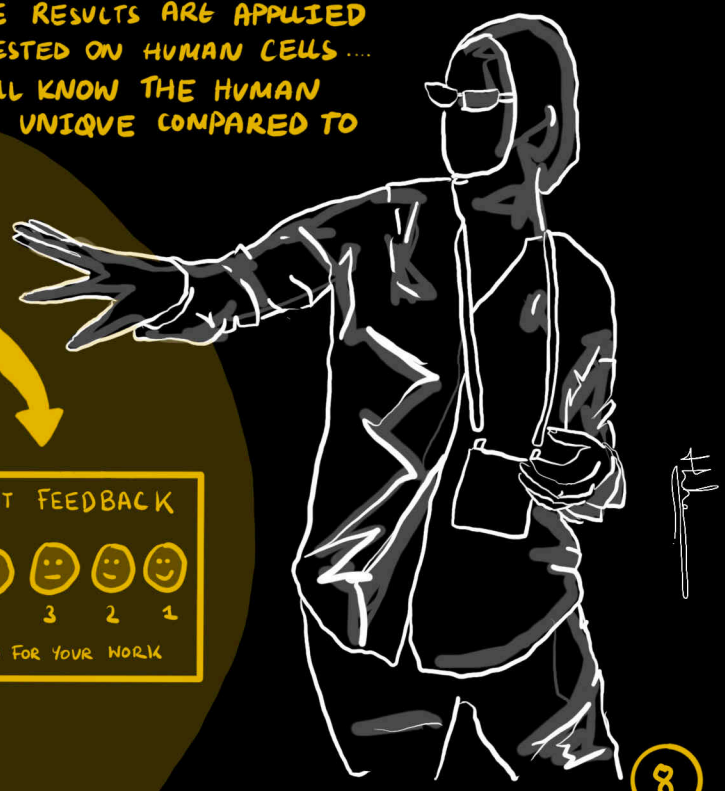
IN-VIVO IS AN EXPERIMENT THATS PERFOMED ON AN ANIMAL (LIKE FISH) OR HUMANS.

IN-VITRO WOULD LOOK AT A PARTICULAR CELL OR A GROUP OF CELLS IN A DISH.

4 SUCCESSFUL RESULTS ON ANIMAL MODELS IS PUBLISHED IN THE SCIENTIFIC COMMUNITY.

SOME OF THESE RESULTS ARE APPLIED FURTHUR AND TESTED ON HUMAN CELLS.... BECAUSE WE ALL KNOW THE HUMAN PHYSIOLOGY IS UNIQUE COMPARED TO ANIMALS.

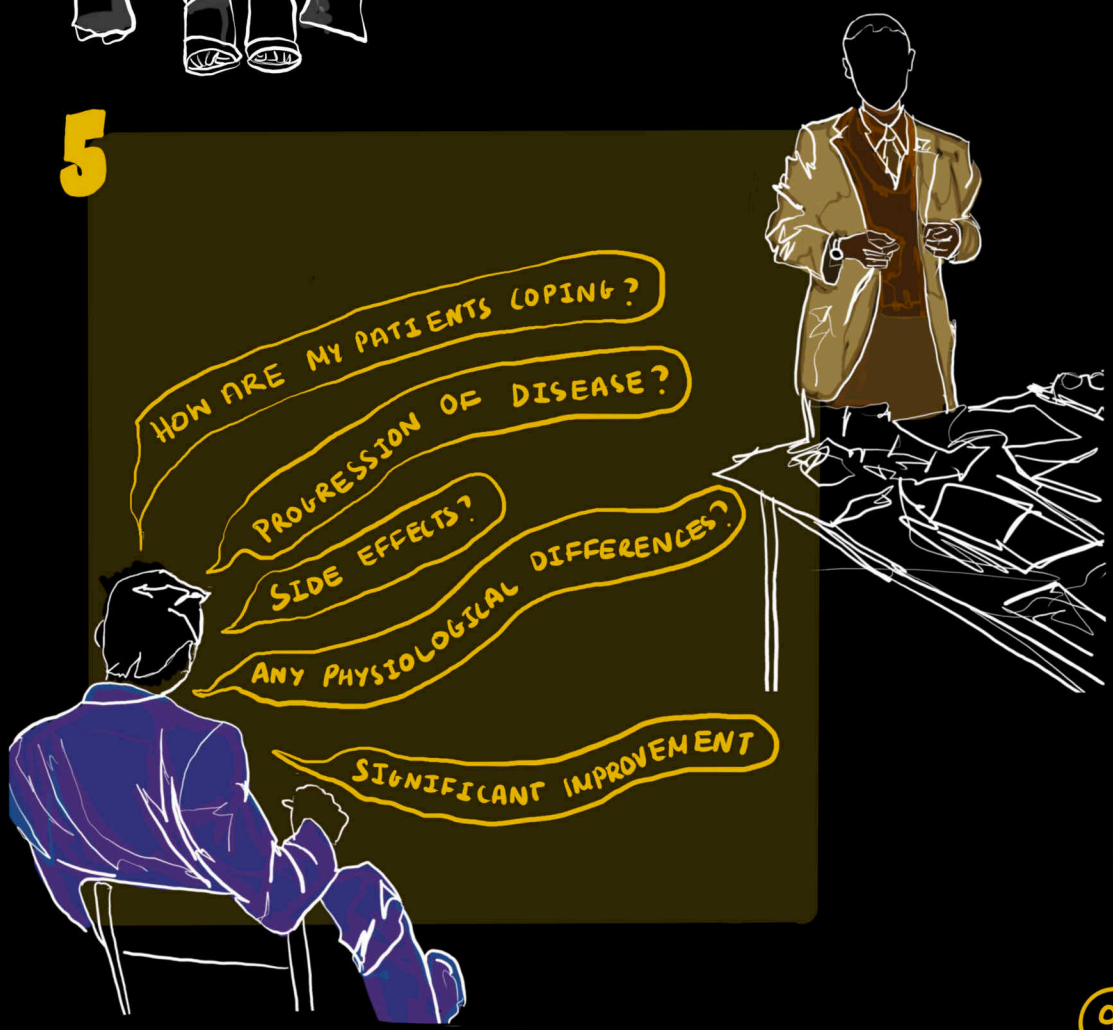




SCIENTIFIC WORK IS NOW APPROVED FOR CLINICAL TRIALS. AT THIS POINT WE ARE FURTHUR INVESTIGATING HUMAN DISEASES.

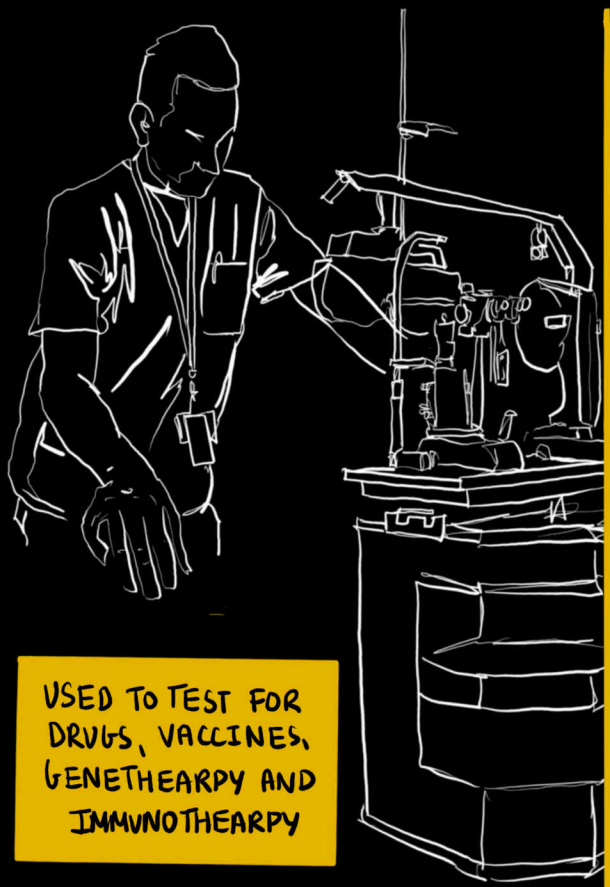
AT THIS POINT ANY APPROVED DRUGS OR THEARPY IS ALSO TESTED FOR COMPATABILITY (ONLY IF APPROVED BY MHRA)

5

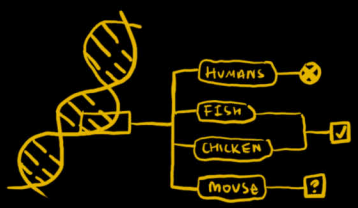


ANIMAL MODELS

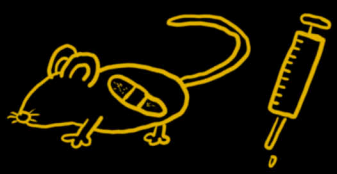
USING ANIMAL MODELS IN RESEARCH IS CONTROVERSIAL BUT MOST RESULTS (WORKING WITH THEM) HAVE SHOWN PROMISING PROGRESSION FOR DISEASES. THAT MEANS TREATMENTS CAN BE AVALIABLE SOONER.



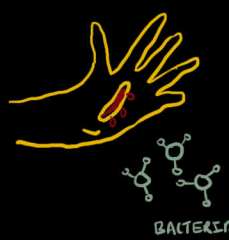
USED TO TEST FOR DRUGS, VACCINES, GENETHEARPY AND IMMUNOTHEARPY

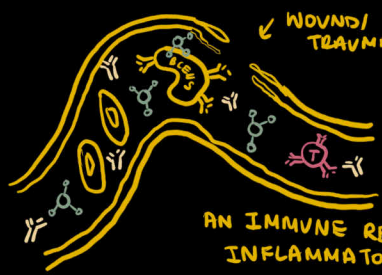


SPECIES ARE CHOSEN ACCORDING TO THEIR GENETIC AND FUNCTIONAL CHARACTERISTICS TO LINE OF RESEARCH E.G KILLIFISH - TO LOOK AT AGE-RELATED RETINAL DISEASES.



UNDERSTAND PATHOLOGY OF DISEASE IN DETAIL





DEVELOP THERAPEUTIC INTERVENTIONS. THIS INCLUDES DRUG THEARPY, IMMUNETHEARPY AND GENE THEARPY.

TOXICITY ?

OPTIMUM DOSAGE ?

SIDE EFFECTS?

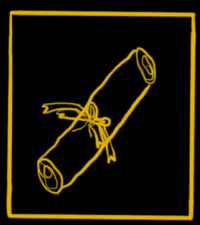
BIOGERONTOLOGY MODELS

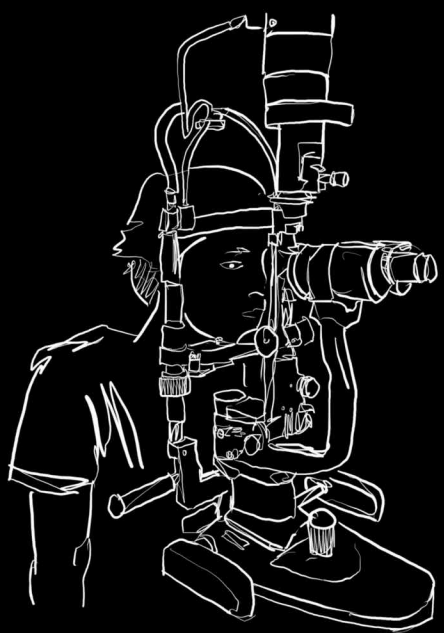
A SUCCESSFUL MODEL COVERS THE CHARACTERISTICS AND FUNCTIONAL CHANGES OF AGE-RELATED DISEASES

...

BUT AT A RAPID PROGRESSION.

MODELS OF RETINAL DISEASES HAVE SHOWN TO IDENTIFY NEW SIGNALLING PATHWAYS INVOLVED IN THE INITIATION AND PROGRESSION OF DISEASE.





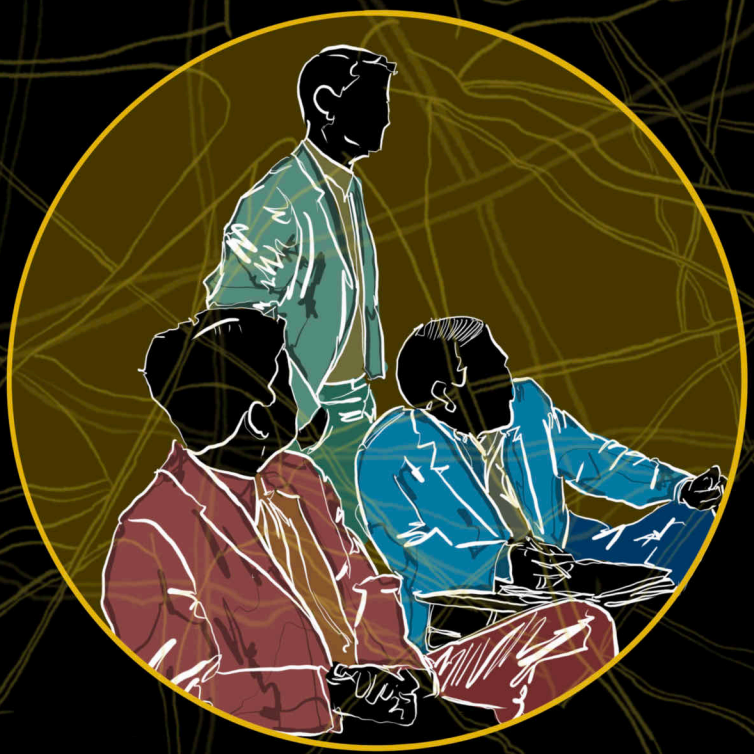
IF BOTH IN-VITRO AND IN-VIVO MODELS SHOW SUCCESSFULL RESULTS. THE RESEARCH IS PROGRESSED INTO CLINICAL TRIALS.

THE PERFECT MODEL DOES NOT EXIST BECAUSE PATIENTS WITH DISEASES SUCH AS AND STILL EXHIBIT PHYSIOLOGICAL CHANGES.

THE AFRICAN TURQUOISE KILLIFISH IS A GREAT MODEL ORGANISM TO UNDERSTAND AGE-RELATED DISEASES. THEIR SHORT LIFE-SPAN OF 16-24 WEEKS. THE KILLIFISH CAN EXHIBIT VARIOUS PHENOTYPES IN SHORT PERIODS OF TIME. STUDIES HAVE SHOWN

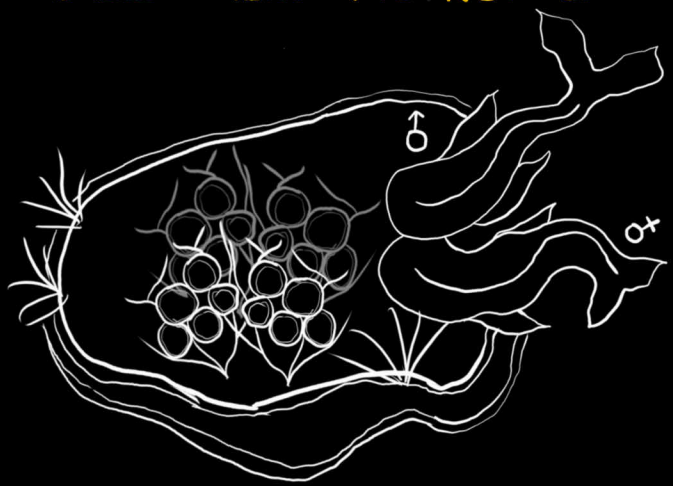
"I CHOOOSE YOU"

THE USE OF AGEING BIOMARKERS CHARACTERISE AGE-RELATED PHENOTYPES. THIS INCLUDES MITOCHONDRIAL INSTABILITY, INCREASE IN APOPTOSIS, COLOUR LOSS AND VISION LOSS.

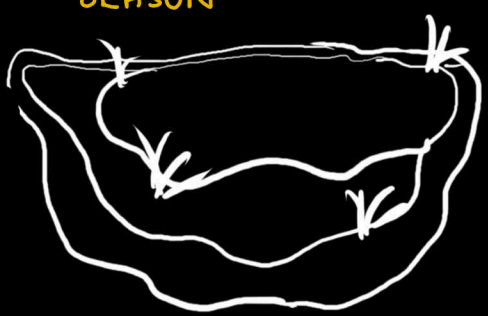


LIFE CYCLE OF KILLIFISH

(1) KILLIFISH NATURALLY LIVE IN EAST AFRICAN PONDS. PAIRS BREED DURING THE WET SEASON.



(2) THE PONDS ARE SUBJECTED TO A BREIF RAINY/WET SEASON FOLLOWED BY A LONGER DRY SEASON



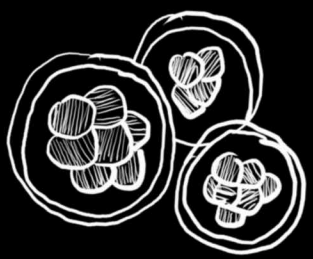
* DIAPAUSE : SUSPENDED DEVELOPMENT IN KILLIFISH IN UNFAVORABLE ENVIRONMENTS

⑦ JUVENILLE KILLIFISH

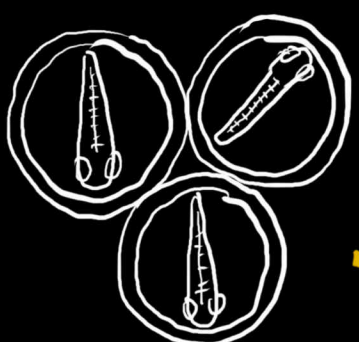
⑥ THE EGGS ARE READY TO HATCH.

THE GOLDEN/YELLOW EYES SHOW WHEN.

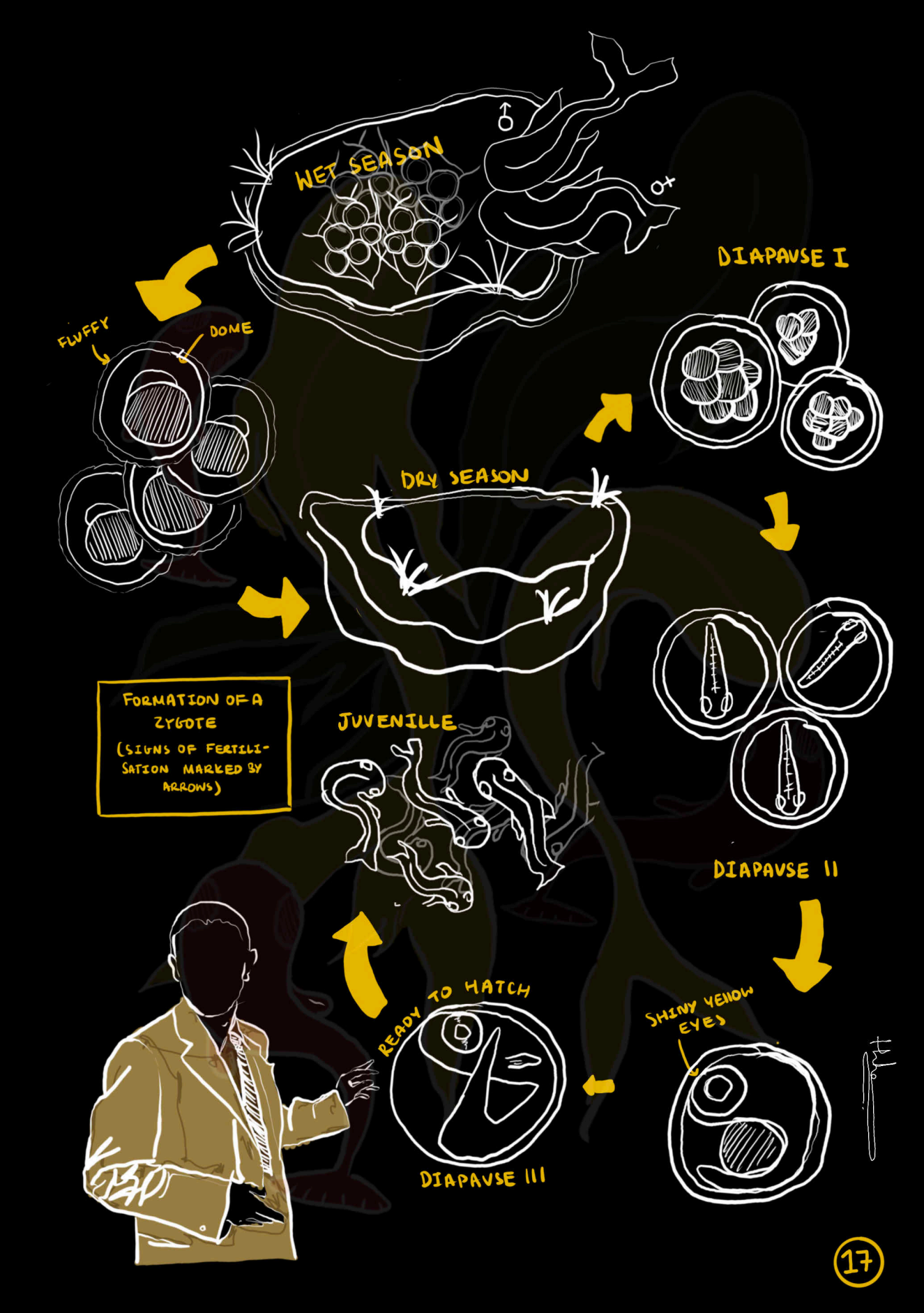
③ DURING THE DRY SEASON, EMBRYOS ENTER *DIAPAUSE



⑤ GROWTH IS DELAYED TO INCREASE SURVIVAL



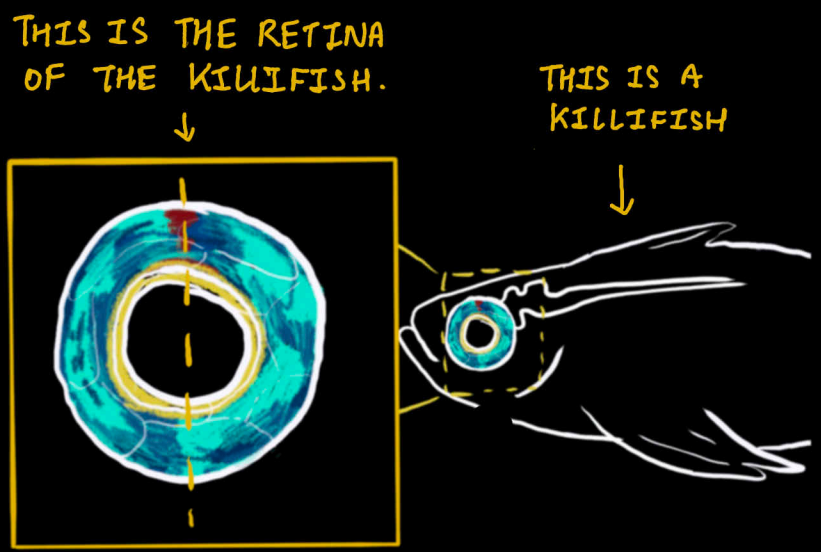
④ KILLIFISH IN DIAPAUSE

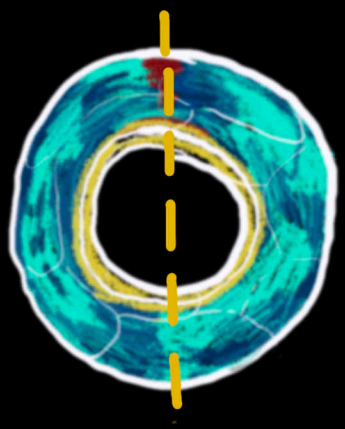

WET SEASON
♂
♀
DIAPAUSE I
FLUFFY
DOME
DRY SEASON
FORMATION OF A ZYGOTE
(SIGNS OF FERTILISATION MARKED BY ARROWS)
JUVENILLE
DIAPAUSE II
READY TO HATCH
SHINY YELLOW EYES
DIAPAUSE III

TO UNDERSTAND WHAT THERAPEUTIC TREATMENT WORKS BEST WITH PATIENTS SUFFERING WITH VISION LOSS. WE NEED TO CHARACTERISE THE CHARACTERISTIC AND FUNCTIONAL CHANGES OF RETINAL DISEASES.

USING KILLIFISH AS A MODEL ORGANISM TO UNDERSTAND AGE-RELATED DISEASES WOULD BE IMPACTFUL. WE STILL HAVE A LONG-WAY TO GO. FIRSTLY WE NEED TO CHARACTERISE THE RETINA OF THE KILLIFISH. WE ALSO NEED TO FIND THE BEST PROTOCOL E.g ANTIBODIES THAT WORK WELL WITH KILLIFISH.

UNDERSTANDING THE STRUCTURE OF THE RETINA AND VISUAL TRANSDUCTION IS IMPORTANT BECAUSE IT ONLY ENHANCES OUR FINDINGS AND LEARNING.



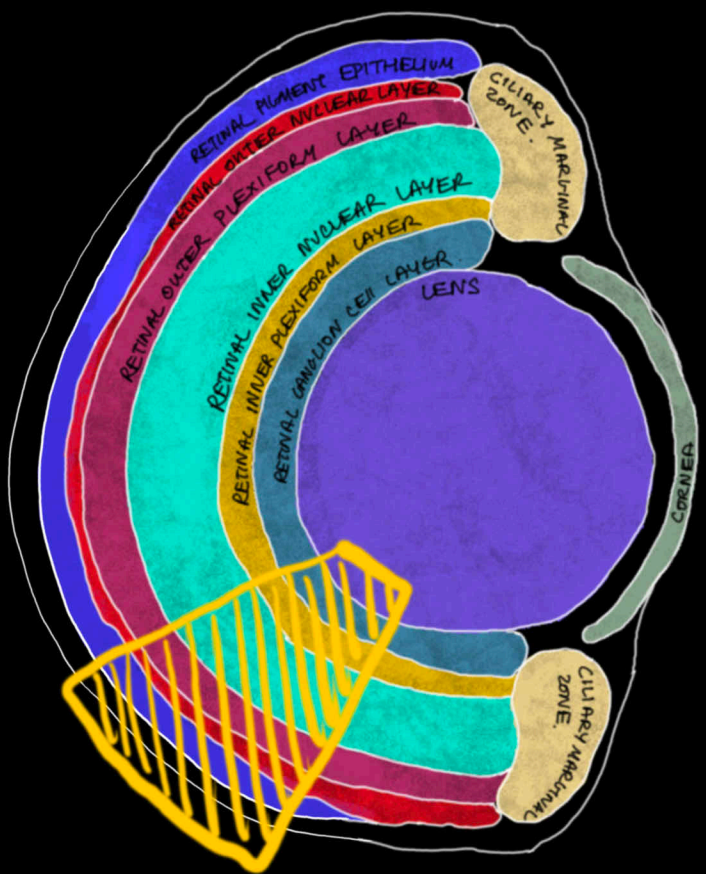


IF WE TAKE THE EYE AND CUT IT IN HALF YOU GET THE CRANIAL CROSS-SECTION OF THE RETINA.

YOU CAN SEE THE LAYERS OF THE RETINA. EACH IS COLOUR-CODED TO ITS ABBREVIATIONS.

EACH LAYER PLAYS A SIGNIFICANT ROLE IN THE FUNCTION OF THE RETINA.

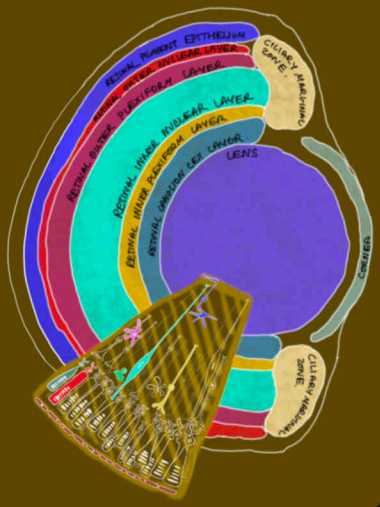
EACH LAYER IN THE RETINA CONTAIN SPECIALISED CELLS. THIS IS WHAT WE WILL LOOK AT NEXT. THE CROSS-SECTION WE WILL LOOK AT IS SHOWN ABOVE.



RPE
ONL
OPI

INL
IPL
GCL

CM2
LENS
CORNEA



THE RETINAL EPITHELIUM LAYER (RPE) IS FOUND ON THE OUTERMOST SURFACE OF THE EYE, FOLLOWED BY THE PHOTORECEPTORS.

WE WILL FOCUS ON PHOTORECEPTORS IN DETAIL LATER ON

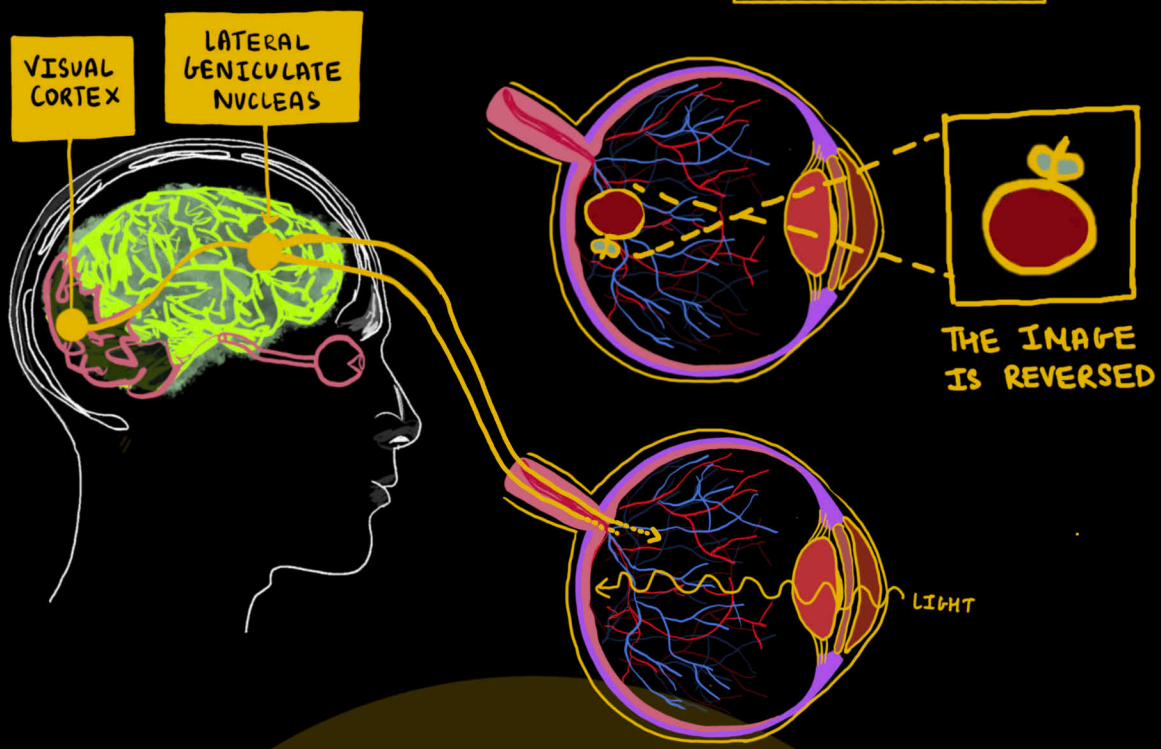
LIGHT TRAVELS FROM THE RPE TO THE RETINAL GANGLION LAYER. WE WILL LOOK AT THIS NEXT.

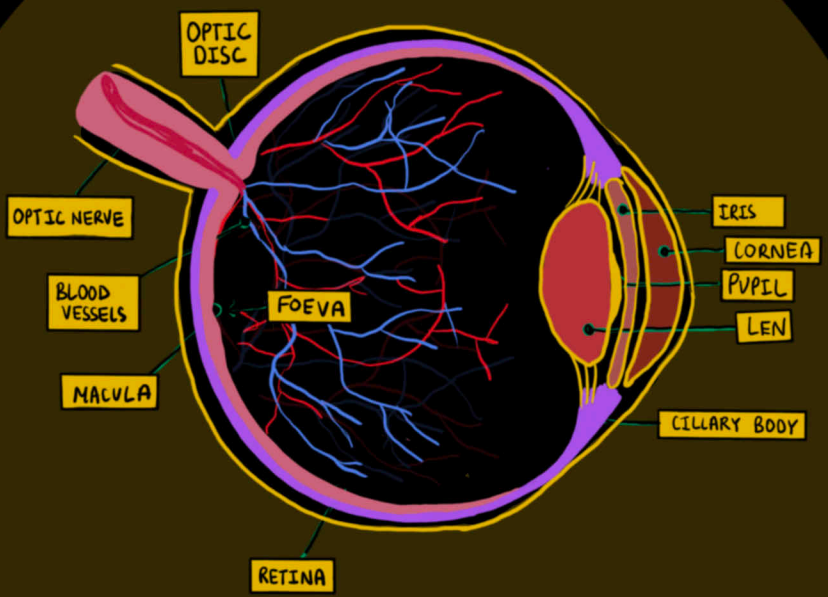
VISUAL TRANSDUCTION

THE CORNEA ALLOWS THE LIGHT TO BEND.

THE IRIS CONTROLS HOW MUCH LIGHT ENTERS THE EYE

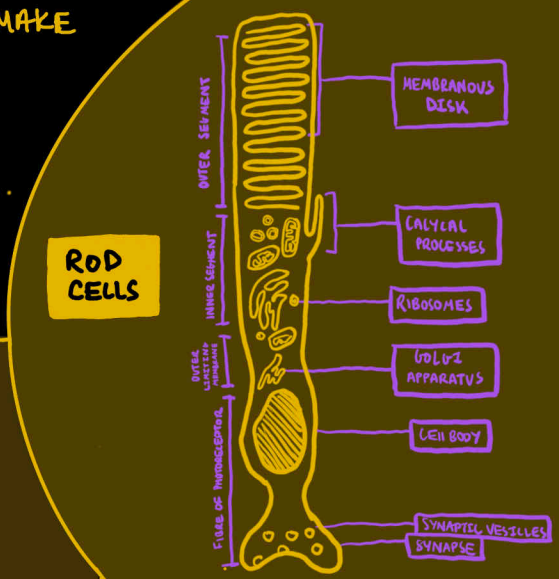
1. FIRST LIGHT PASSES THROUGH **CORNEA** SOME OF THIS LIGHT ALSO ENTERS THE PUPIL.

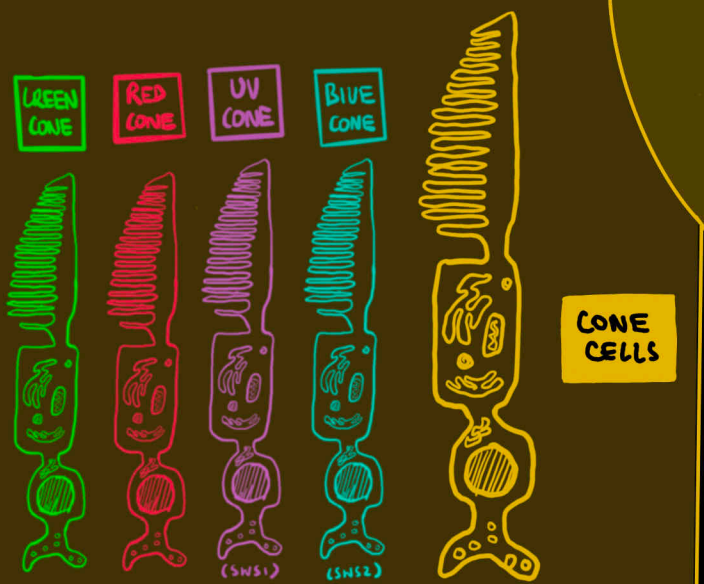




② LIGHT HITS THE RETINA. THE RETINA IS PACKED WITH PHOTORECEPTOR. THESE CELLS ARE SPECALISED BECAUSE THEY ARE LIGHT SENSITIVE. THE PROTEIN LIGHT PIGMENTS THEY CONTAIN, MAKE THEM UNIQUE (SEE TABLE BELOW)

THEY WORK BY CONVERGING LIGHT SIGNALS INTO ELECTRICAL SIGNALS. WE WILL GO INTO THE DETAILS OF THIS IN THE NEXT PAGE.

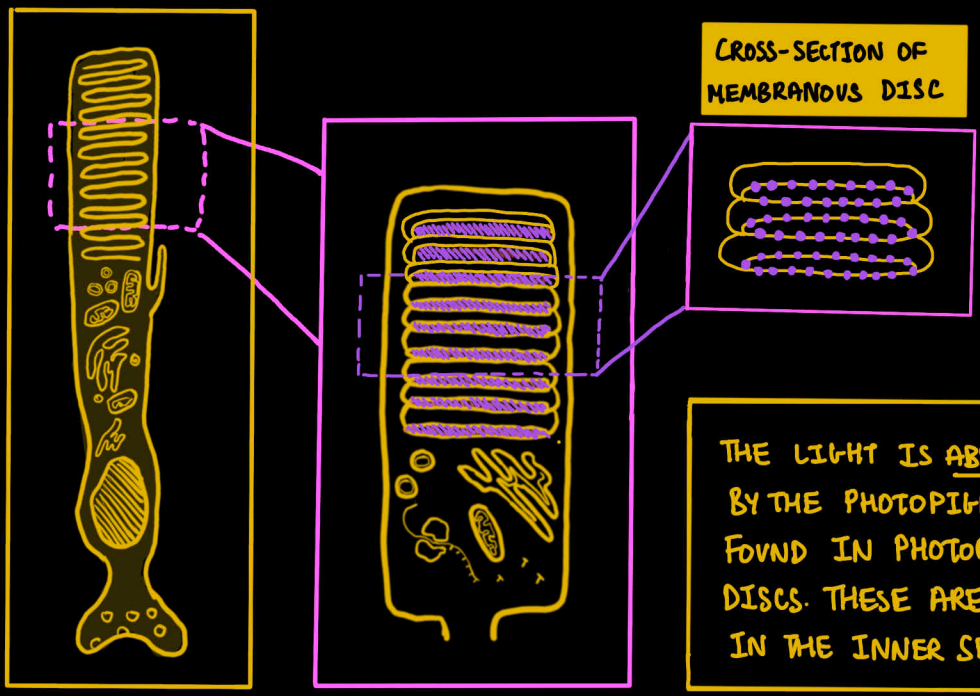




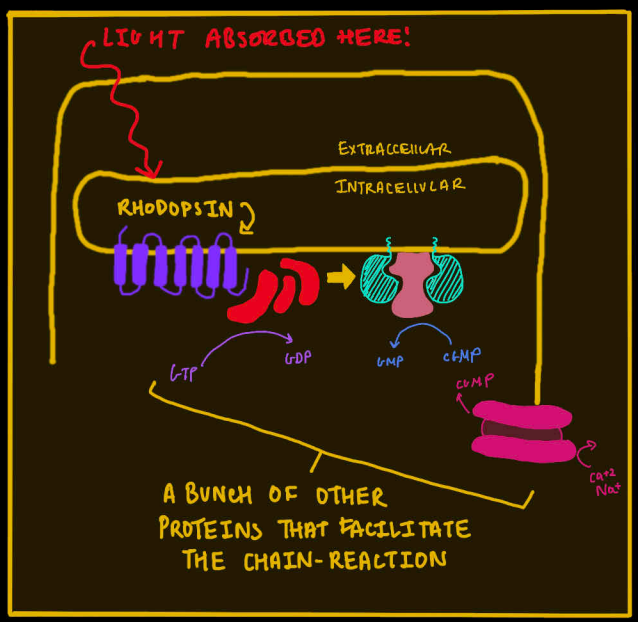
THE CONE HAS 4-SUBTYPES.

THE TABLE BELOW SHOWS THE PROTEIN PIGMENTS

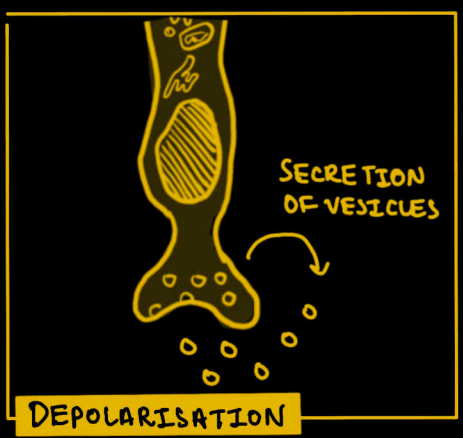
	PIGMENT MOLECULE			
RODS	RHODOPSIN			
CONES	BLUE CONE	RED CONE	UV CONE	GREEN CONE
	BLUE OPSIN	RED OPSIN	UV OPSIN	GREEN OPSIN



THE LIGHT IS ABSORBED BY THE PHOTOPIGMENTS FOUND IN PHOTORECEPTOR DISCS. THESE ARE FOUND IN THE INNER SEGMENT.



1. THE VISUAL PIGMENTS ARE EMBEDDED INTO THE MEMBRANOUS DISC.
2. THE LIGHT ABSORBED TRIGGERS A CONFORMATIONAL CHANGE
3. THIS INITIATES A SERIES OF EVENTS THAT TRIGGER A POTENTIAL.



THE DEPOLARISATION CLOSES THE SODIUM CHANNEL. THIS HYPER-POLARISES THE CELL.

OUR OBJECTIVES FOR THIS EXPERIMENT WAS TOO :

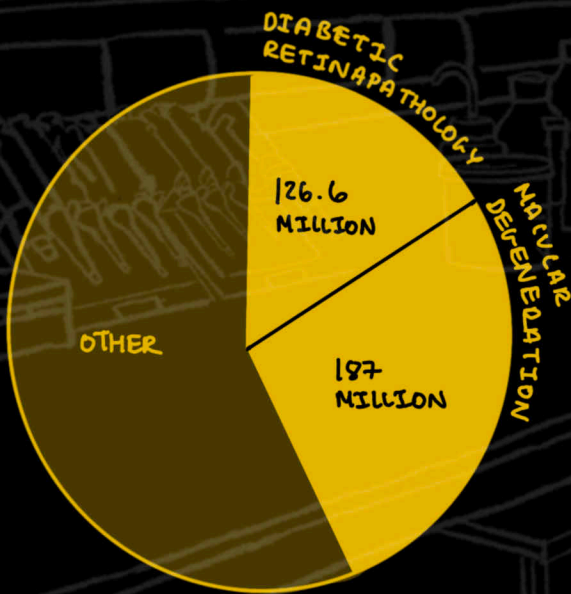
- CHARACTERISE KILLIFISH RETINA USING mRNA AND PROTEIN EXPRESSION VIA **IMMUNOHISTOCHEMISTRY**
- ASSESS GENETIC COMPONENTS OF PHOTOTRANSDUCTION GENES VIA **IN-SITU HYBRIDISATION**

THESE WERE PERFORMED ON 4-12 WEEK KILLIFISH

FROM THIS WE WERE ABLE TO:

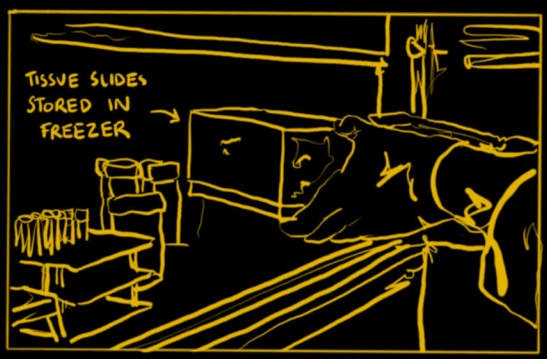
- ASSESS GENETIC COMPONENT OF PHOTORECEPTOR OPSIN GENES
- VISUALISE THE LOCALISATION OF DIFFERENT GENETIC PROBES IN DIFFERENT RETINAL CELL-TYPES

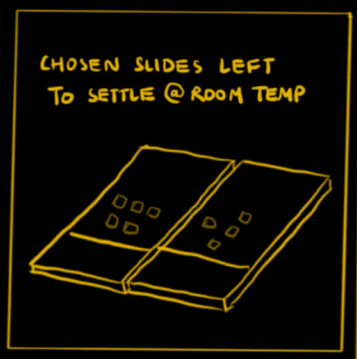
OUR FOCUS WILL BE ON PHOTORECEPTORS BECAUSE DISEASES CAUSED BY VISION LOSS IS COMMON IN AGE-RELATED DISEASES

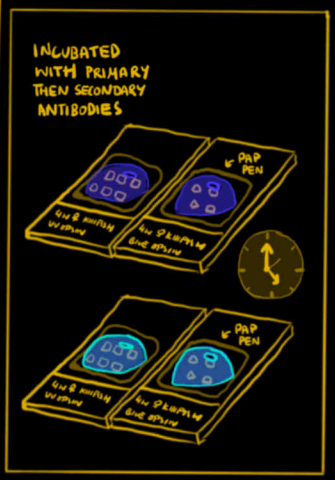


IN THE NEXT FEW PAGES WE WILL GO THROUGH EXPERIMENTAL TECHNIQUES INCLUDING **IHC** AND **HCR**

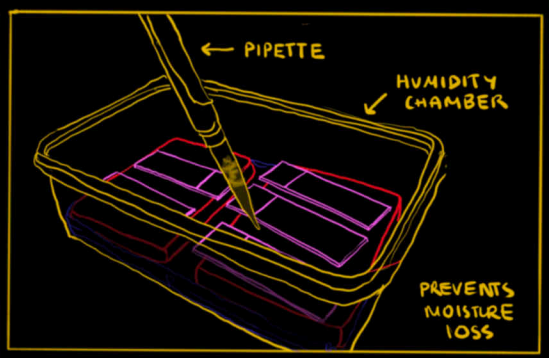
IMMUOHISTO

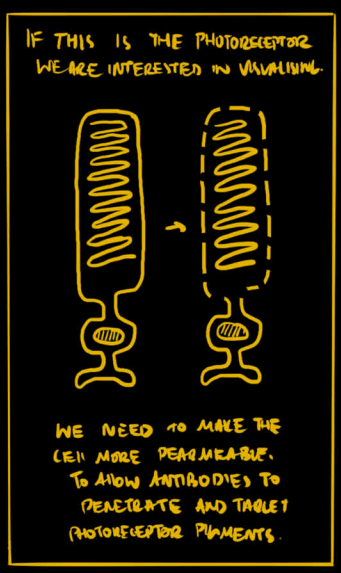

TISSUE SLIDES STORED IN FREEZER

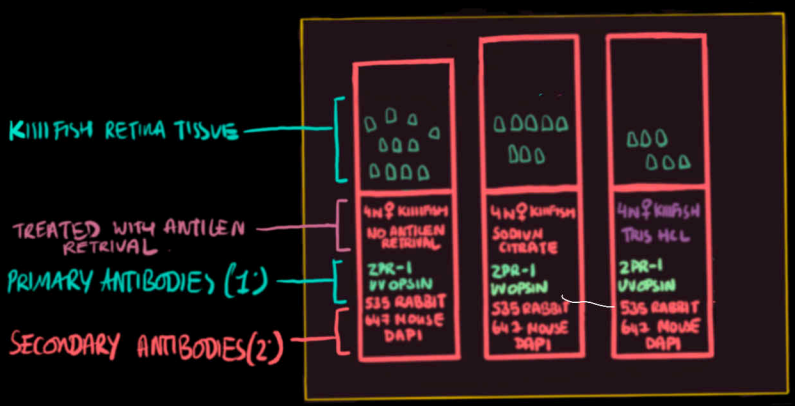

CHOSEN SLIDES LEFT TO SETTLE @ ROOM TEMP


INCUBATED WITH PRIMARY THEN SECONDARY ANTIBODIES
PAP PEN
PAP PEN

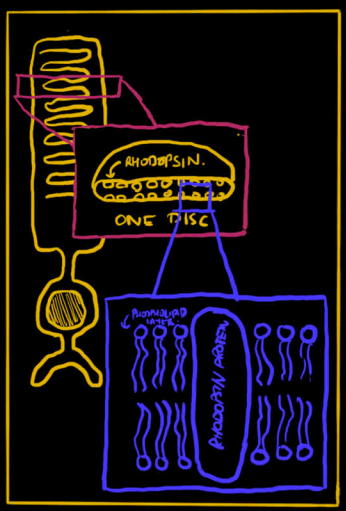

REHYDRATION & FIXATION OF CELLS. THIS PRESERVES INTEGRITY OF TISSUE
BLOCKING SERUM PREVENTS NON-SPECIFIC BINDING

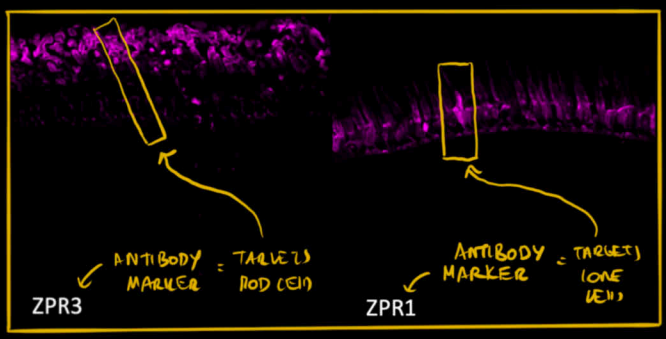

PIPETTE
HUMIDITY CHAMBER
PREVENTS MOISTURE LOSS

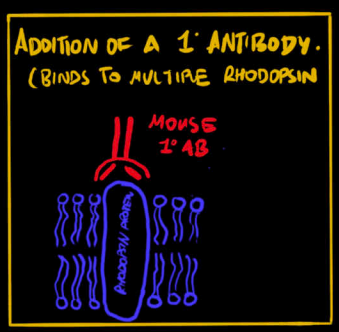

IF THIS IS THE PHOTORECEPTOR WE ARE INTERESTED IN VISUALISING.
WE NEED TO MAKE THE CELL MORE PERMEABLE. TO ALLOW ANTIBODIES TO PENETRATE AND TARGET PHOTORECEPTOR PIGMENTS.

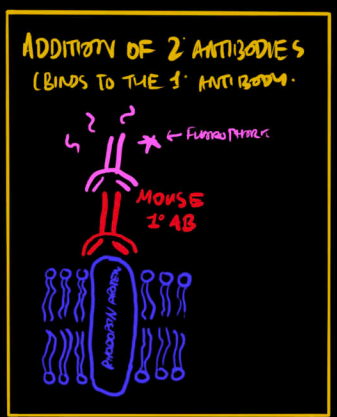

KILLIFISH RETINA TISSUE
TREATED WITH ANTIGEN RETRIVAL
PRIMARY ANTIBODIES (1°)
SECONDARY ANTIBODIES(2°)
4N♀ KILLIFISH
NO ANTIGEN RETRIVAL
2PR-1 RH OPSIN 535 RABBIT 647 MOUSE DAPI
4N♀ KILLIFISH
SODIUM CITRATE
2PR-1 RH OPSIN 535 RABBIT 643 MOUSE DAPI
4N♀ KILLIFISH
TRIS HCL
2PR-1 RH OPSIN 535 RABBIT 643 MOUSE DAPI

CHEMISTRY





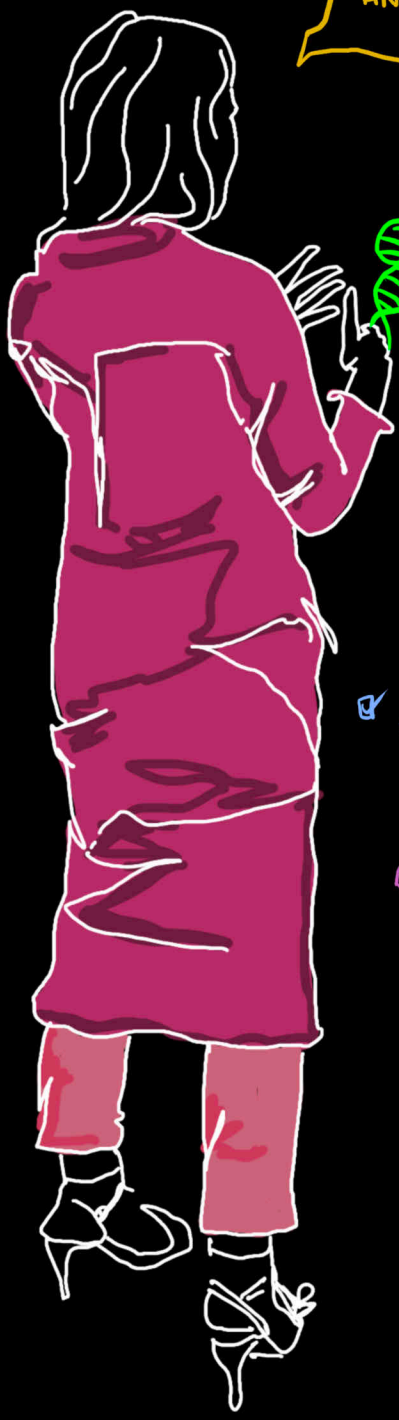


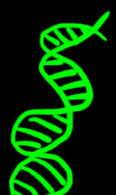


IHC ROUNDS	ANTIBODIES USED	CELL-TYPE
ROUND ONE	ZPR1	DOUBLE RED/GREEN CONES
	ZPR3	RHODOPSIN IN RODS AND GREEN CONES
ROUND TWO	UV-OPSIN	UV CONES
	BLUE-OPSIN	BLUE CONES
ROUND THREE (DIFFERENT PROTOCOL)	UV-OPSIN AND ZPR1	
	BLUE OPSIN AND ZPR3	

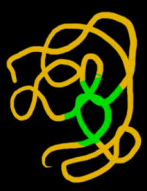
HCR

IN SITU HYBRIDISATION (ALSO KNOWN AS HCR) ALLOWS US TO STUDY GENE EXPRESSION AND GENE PRODUCT . IN PARTICULAR THOSE THAT WE ARE INTERESTED IN





DNA



PROTEIN AGGREGATE

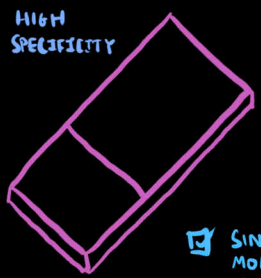
→ WHAT DOES IT DO?

→ HOW IS THIS PROTEIN REGULATED?

→ HOW DOES IT BEHAVE IN DISEASE?

ALL THESE INFORMATION PROVIDE US WITH INFORMATION ON PHENOTYPIC FUNCTION AND GENE REGULATION

☑ HIGH SPECIFICITY



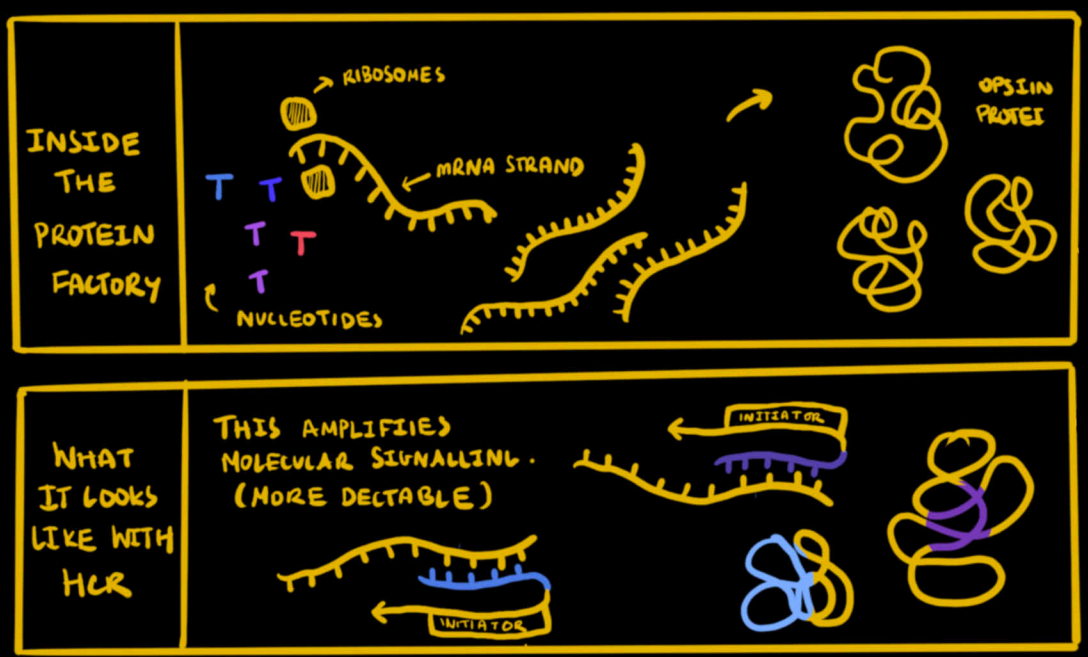
☑ SINGLE MOLECULE SENSITIVITY

→ HCR ON KILLIFISH HAVE SHOWN HIGH SPECIFICITY AND SINGLE MOLECULE SENSITIVITY

SO, HOW DOES A HCR WORK?

THE MRNA OF INTEREST IS IMAGED HCR WORKS BY AMPLIFYING MRNA EXPRESSION – TO BOOST FLUORESCENCE SIGNALING.

OUR AIM WITH IN-SITU HYBRIDISATION (HCR) IS TO VISVALISE SPECEFIC CELL TYPES IN THE RETINA. THIS IS DIFFERENT FROM IMMUNOHISTOCHEMISTRY BECAUSE IT TARGETS MRNA.



MULTIPLEXED MRNA SIGNALLING IN TISSUE-MOUNTS OR WHOLE MOUNT KILLI-FISH GIVES US DISTINCT FLUROPHORE IMAGING

① HYBRIDISE PROBE SET AND WASH.

(YOU CAN DO MULTIPLE). RNA PROBE GUCY2D GNAT1

① THE SPLIT INITIATOR PROBES EACH CARRY AN INITIATOR. THIS STRAND THEN BINDS ONTO THE TARGETTED mRNA.

② ANY UNUSED PROBES ARE WASHED AWAY.

THESE SPLIT INITIATOR RNA PROBES HYBRIDISE TO mRNA SPECIFICALY!

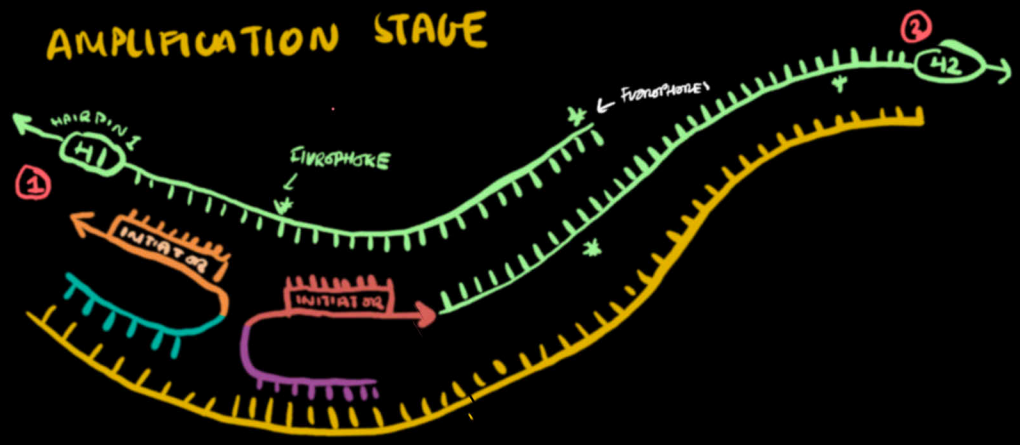
②

THE HAIRPINS DRIVE THE ASSEMBLY CASCADE TO AN RNA INITIATOR SEQUENCE

② IN TURN HYBRIDISING INTO THE INPUT DOMAIN OF H2

① PROBE SETS HYBRIDISE TO mRNA TARGETS TO FORM A DOUBLE STRAND.

(3) AMPLIFICATION STAGE



(1) HYBRIDISATION OF H1 TO INITIATOR LEAVES THE H1 OUTPUT DOMAIN EXPOSED.

(1) SPECIFICALLY BOUND PROBES TRIGGER THE SELF-ASSEMBLY OF FLUORESCENT AMPLIFICATION POLYMERS (THE HAIR-PINS LABELLED AS H1 AND H2)

(2) ANY UNUSED HAIRPINS ARE WASHED AWAY.

THE RNA PROBES WE LOOKED AT ARE LISTED BELOW ON A TABLE

THE GREEN IDENTIFIES SUCESSFULL LOCALISATION

DON'T FORGET, THESE ARE RNA PROBES WE ARE INTERESTED IN

THE RNA PROBES CAN ALSO BE CALLED INITIATORS

INITIATOR	CELL TYPES					
	BIPOLAR CELLS			MICRO-GLIAL	PHOTORECEP-TORS	HORIZON-TAL
	1	2	3			
B1	elna	nxph1	olx2	metap2	gnat1	isl1
B2	Fezl2	neurod2	Pvalb9	mHla	gnat 2	hmx2
B3	Sebox	dmbx1b	nxph2	Slc7a2	gucy20	Barhl2

THIS WAS COMPARED TO A CONTROL OF PROBES, THAT HAVE SHOWN TO WORK WELL IN PREVIOUS STUDIES

EACH OF THESE PROBES PLAY A SIGNIFICANT ROLE IN THE RETINA.

HOWEVER, FOR NOW WE ARE ONLY INTERESTED IN VISUALISING IT.

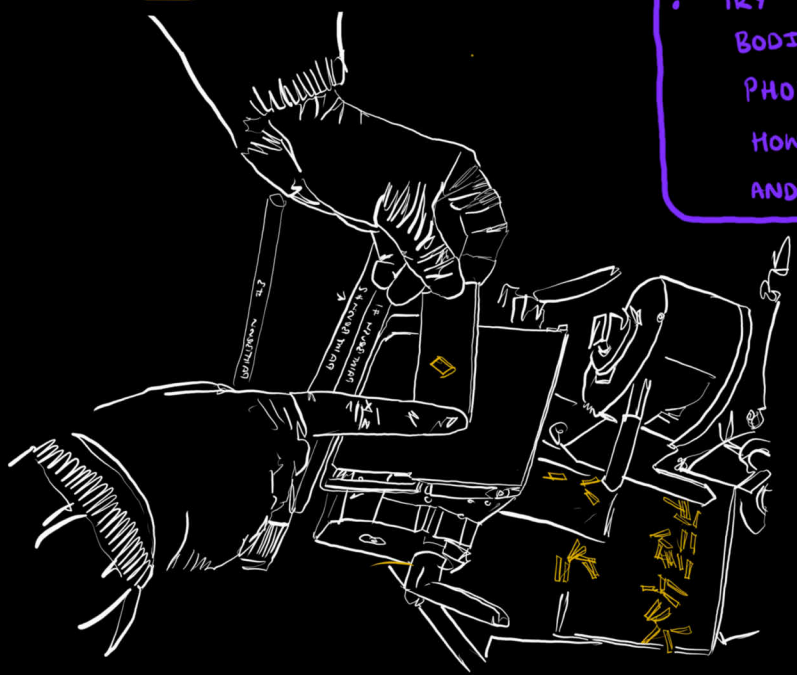
THERE ARE MANY DIRECTIONS WE CAN TAKE TO UNDERSTAND RETINAL DISEASES USING KILLI-FISH AS FISH MODELS IS ONE OF THEM. AS WELL AS PERFECTING PROTOCOL.

THE AGE OF ONSET INFLUENCES MANAGEMENT OF DISEASE

HENCE I WOULD LOOK AT THE RETINAL GENES IN DIFFEREN KILLIFISH AGE GROUPS

WHAT MORE?

- WE WILL CHALLANGE AND TRIAL VARIOUS PROTOCOL TO ACHIEVE BEST RESULTS
- TRY VARIOUS OTHER ANTI-BODIES (SPECIFIC TO PHOTORECEPTORS) AND SEE HOW THEY WORK IN HEALTHY AND DISEASED KILLIFISH
- DEVELOP THERAPEUTIC INTERVENTIONS TO TREAT RETINAL DISEASES.



SPECIAL THANKS

DR NICOLE NOEL

NICOLE CHIU

AANANDITA KOTHURKAR

DR MANUELA LAHNE

DR RYAN MACDONALD

DR ARTUR R FERNANDES

NATALIA JAROSZYNSKA

XHULJANA DURNISHI

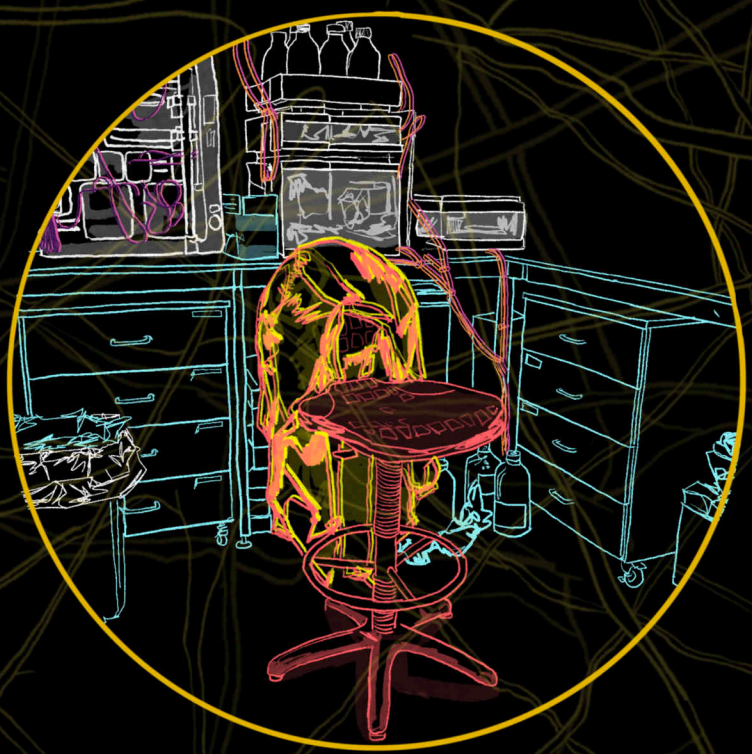
GREGORY PATIENT

HUALIN YI

giantjelly23@gmail.com

GiantJelly2_3

ZEEKS
Art for Geeks
Tranforming The Way You Think About Data.



In²science UK

ZEBRAFISH UCL

UCL