



Räumliche Orientierung als Fenster in die Funktion des Gehirns

Modellsysteme in der Neurobiologie — NWG Lehrerfortbildung 2017/2018
Dr. Matthias Wittlinger

Räumliche Orientierung – Raumsinn oder Richtungssinn

Fähigkeit von Menschen und Tieren, die ihnen hilft, sich im Raum und richtungsbezogen zurechtzufinden und angemessen zu bewegen.



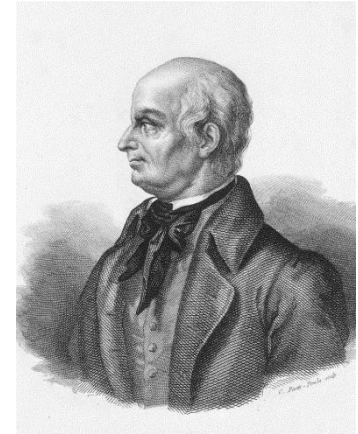
Heimfindevermögen – homing behavior

Beispiele im gesamtem Tierreich



Experimentelle Biologie – Beispiel Echoortung

- 1773 Lazzaro Spallanzani
- 1778 Charles Jurine
- 1938 Robert Galambos und Donald Griffin
Ultraschalldetektor von William Pierce



Quelle: Wikipedia



Santschi's Spiegelexperiment *Messor barbatus*

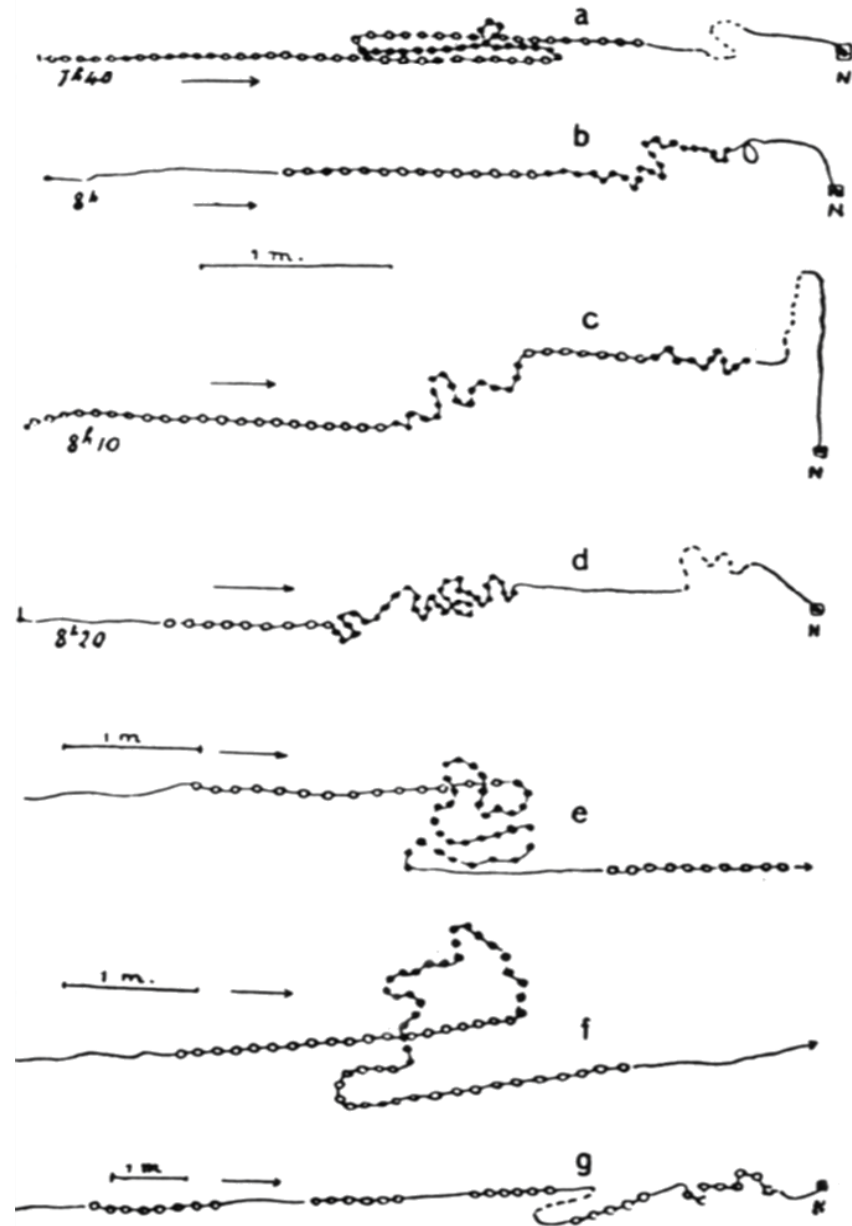
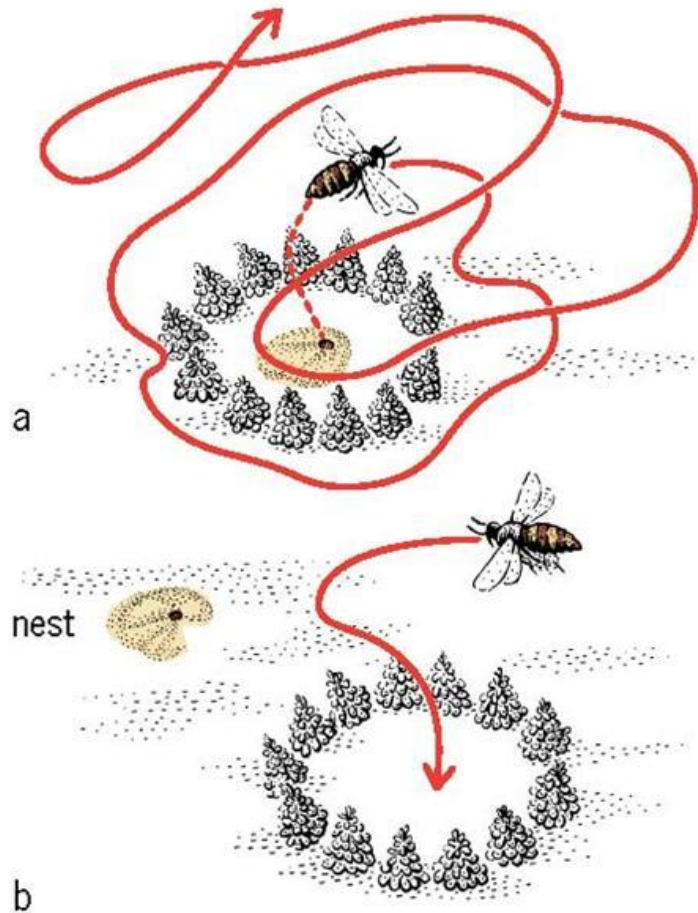
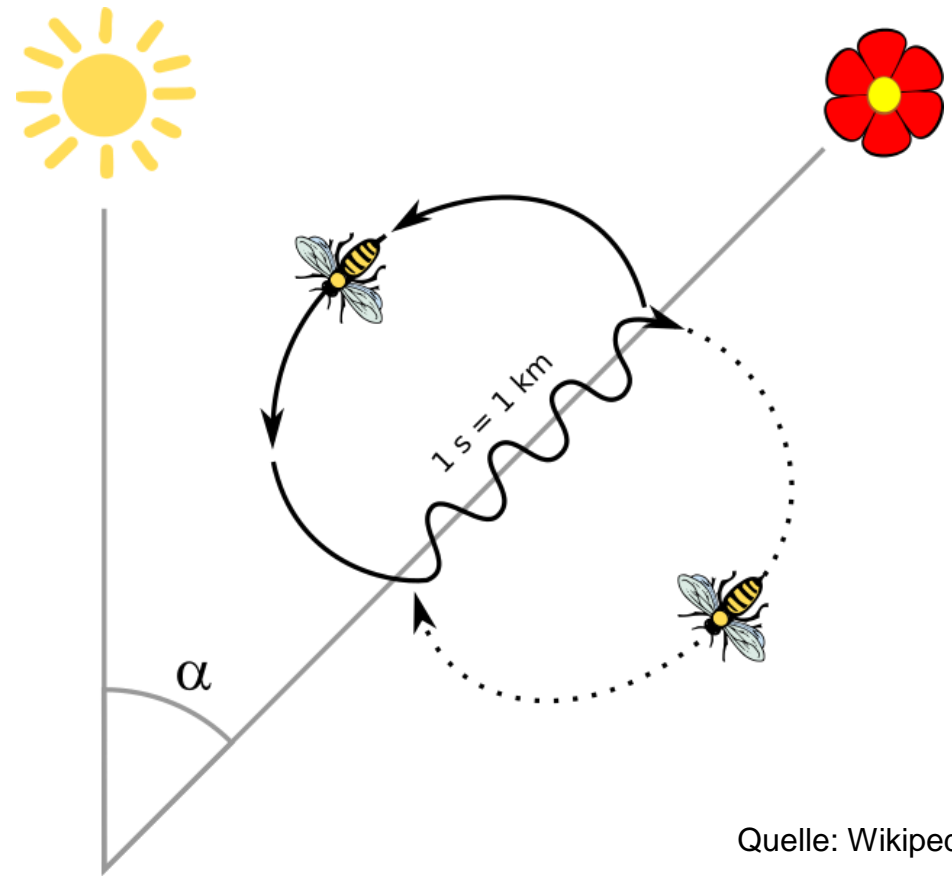


FIG. 2.

Santschi (1911; 1923)



Nikolaas Tinbergen



Quelle: Wikipedia

Karl von Frisch

Nobelpreis 1973: „für ihre Entdeckungen zu Organisation und Auslösung von individuellen und sozialen Verhaltensmustern“



Warum Forschung an Wüstenameisen?

landmarkenarm

lebensfeindlich: heiß, trocken, salzig

wenig Nahrung

- keine Duftspuren
- tagaktive Einzelfuragierer
- kein Rekrutieren von Nestgenossen

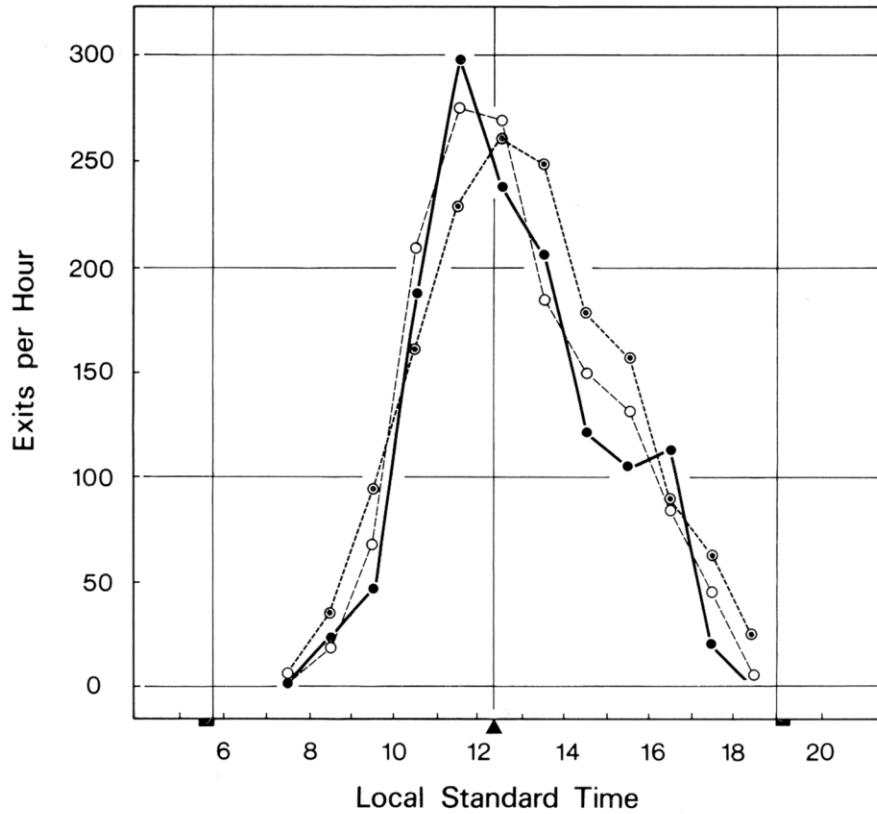


Lebensraum von *Cataglyphis fortis*



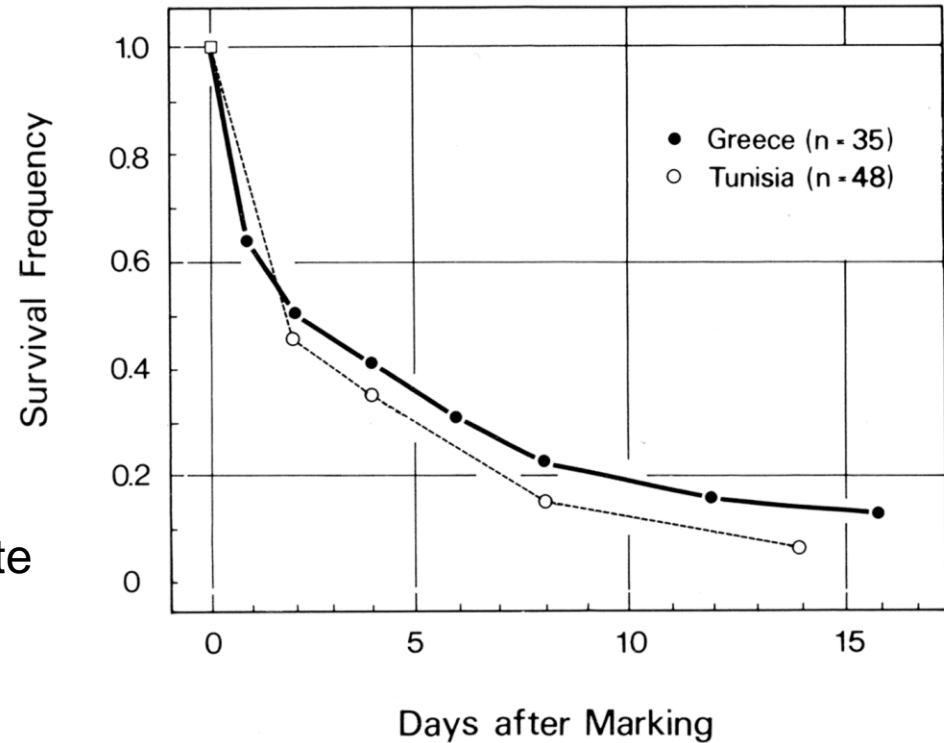
viele Raubfeinde





Aktivitätspeak um Mittag

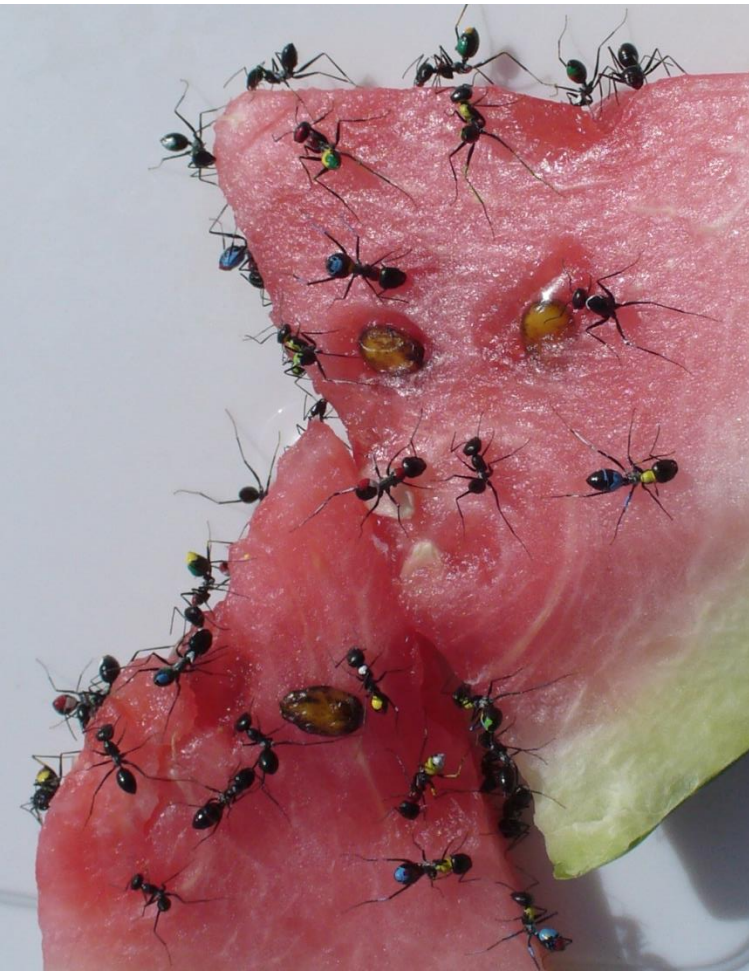
Niedrige Überlebensrate
(ca. 50% nach 2d)

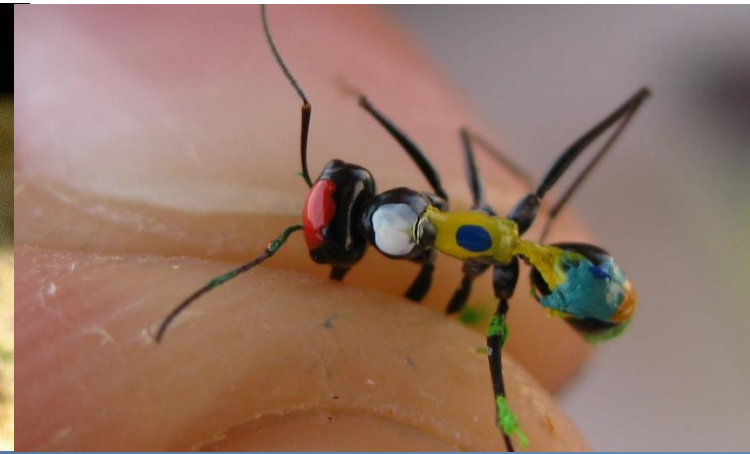




Experimentelle Vorteile von *Cataglyphis* Ameisen:

- direkt beobachtbar (cf. Bienen, Tauben, etc.)
- individuelle Identifikation
- einfach zu Manipulieren



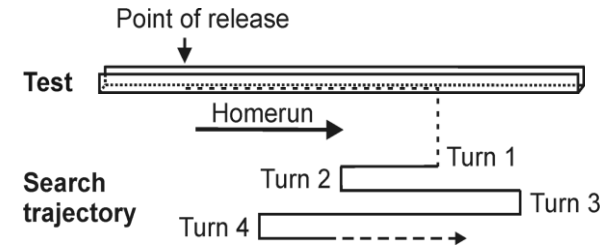
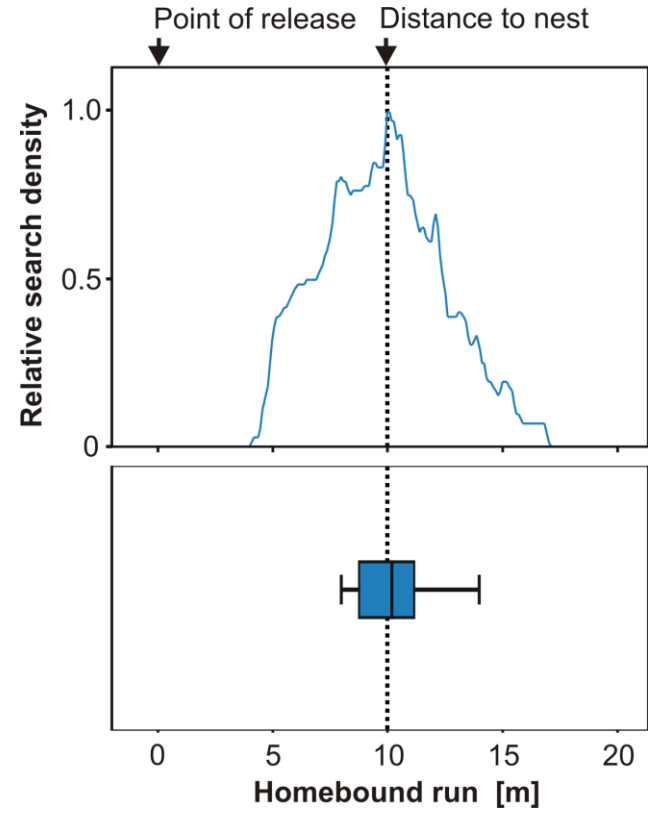
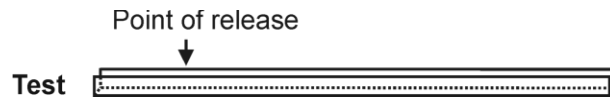
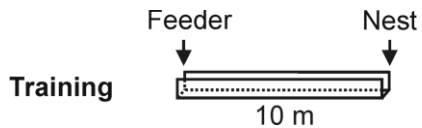
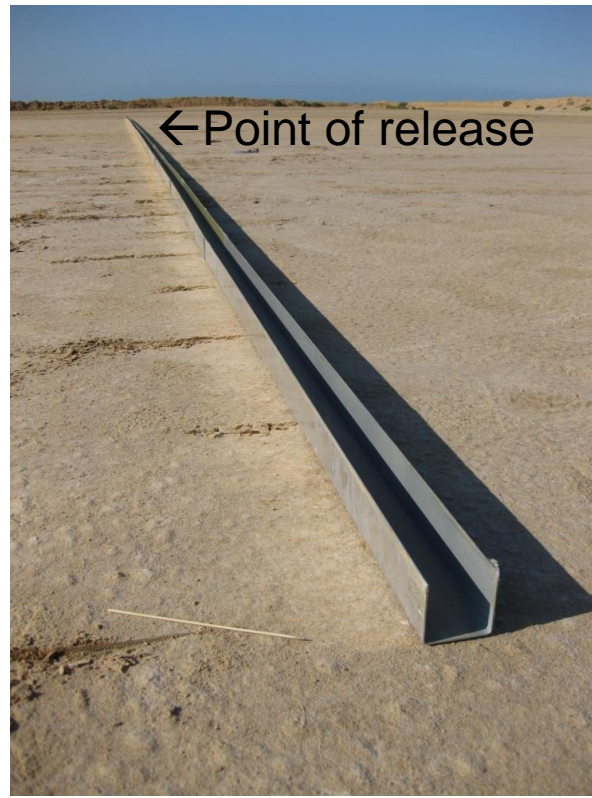
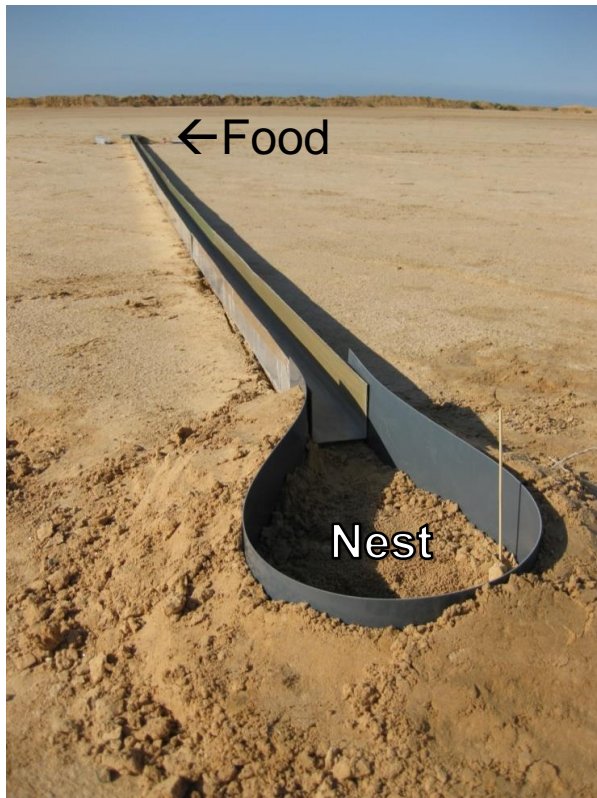


Landmarkenarme Testfelder

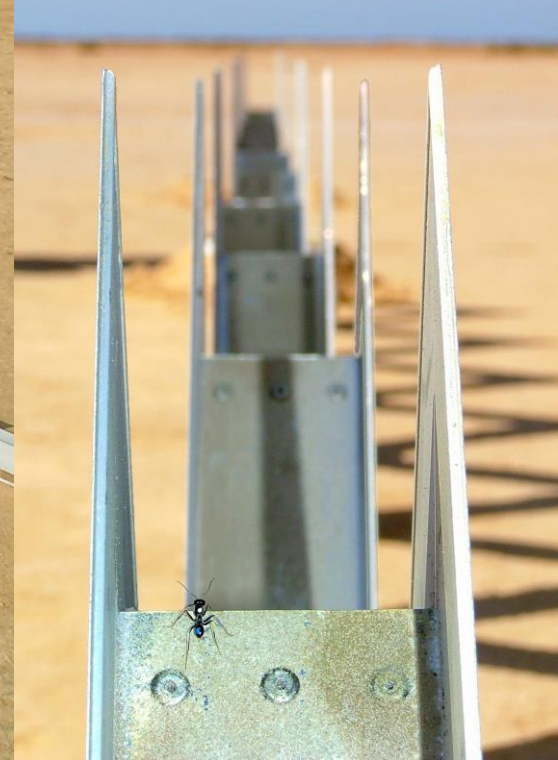
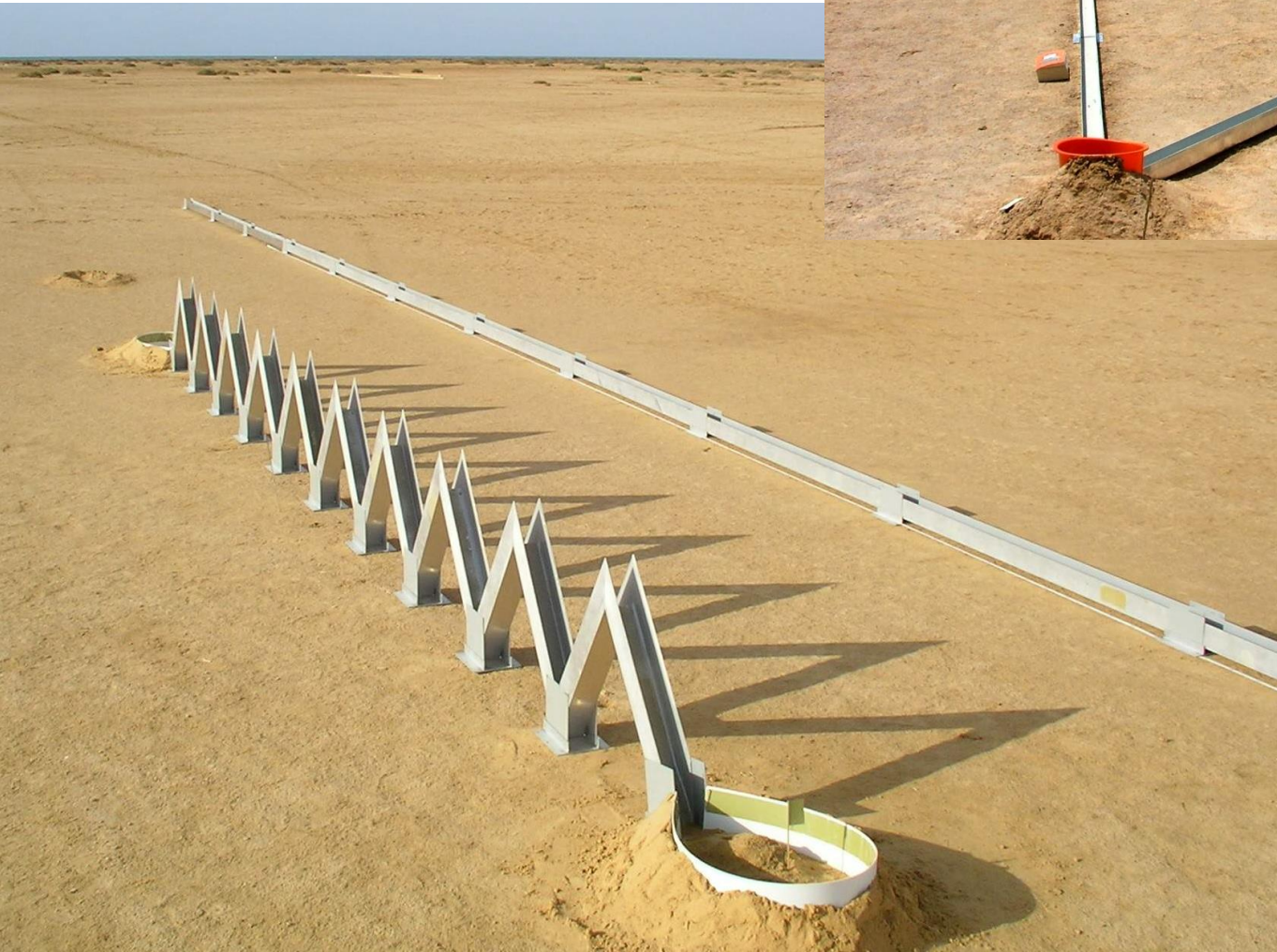


from Wehner 1983

Kanalexperimente 1D

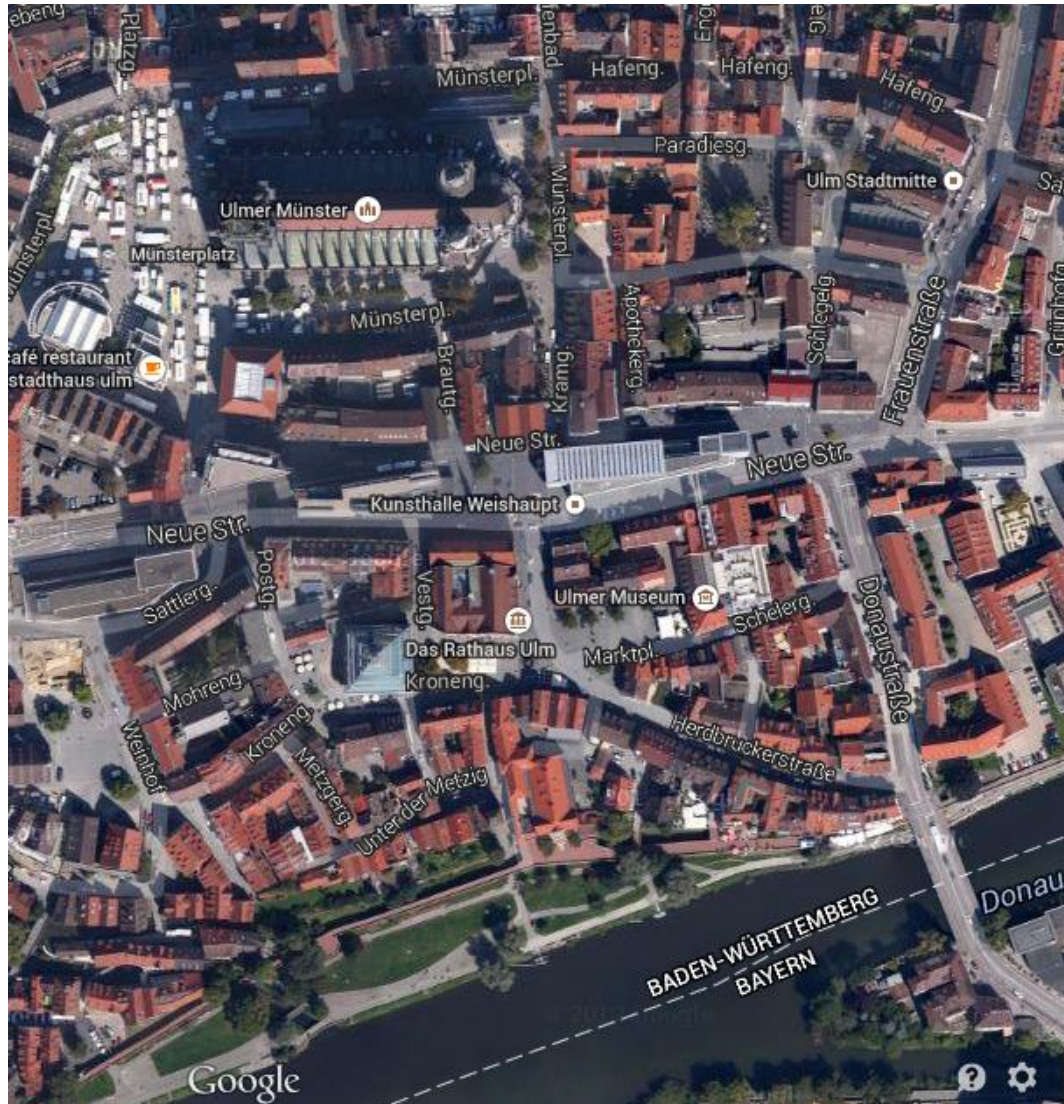


Kanalexperimente 2D and 3D

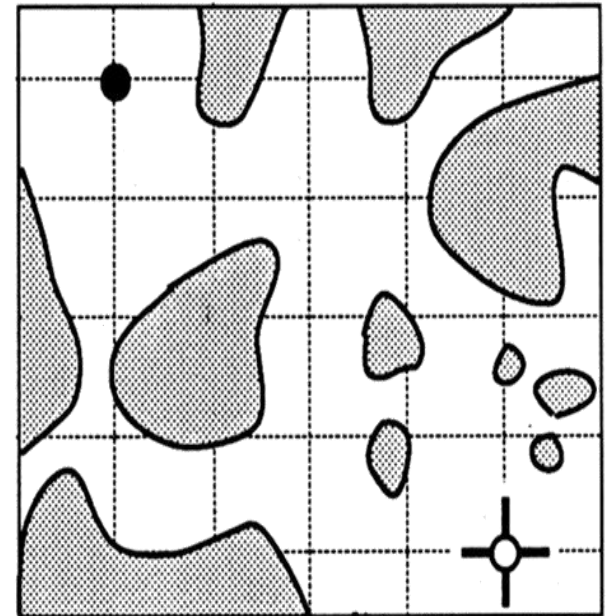


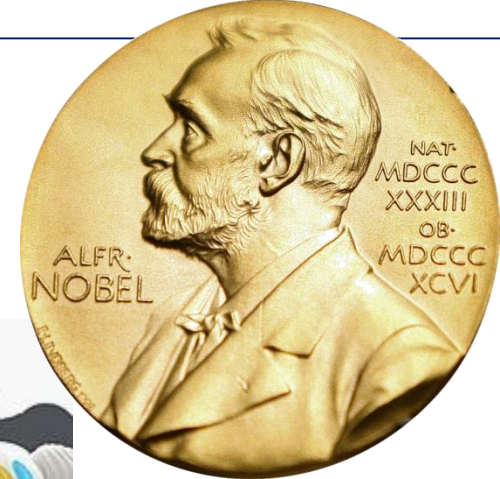


Grundsätzliche Navigationsstrategien

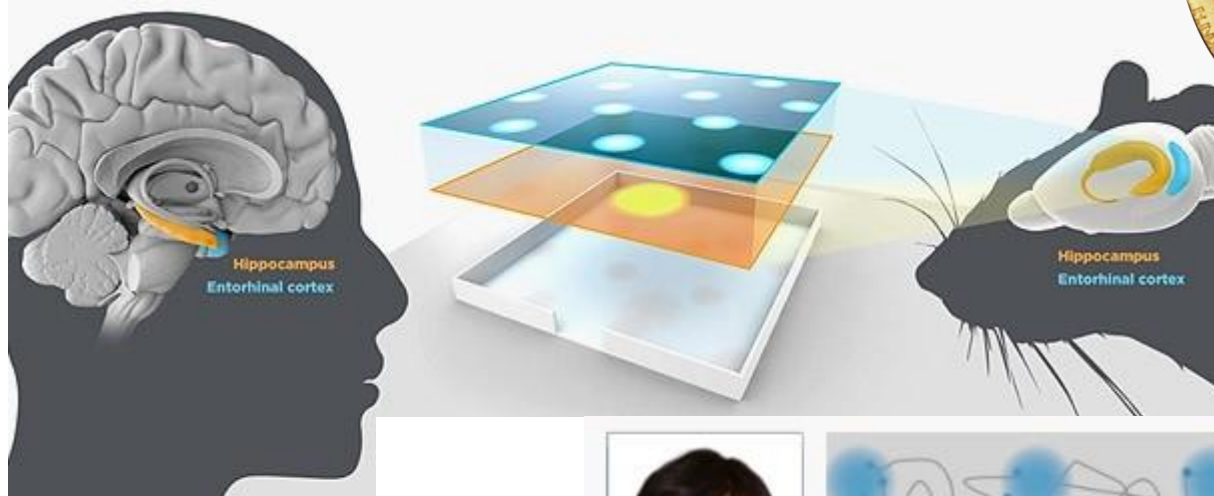


kartenbasierte Systeme

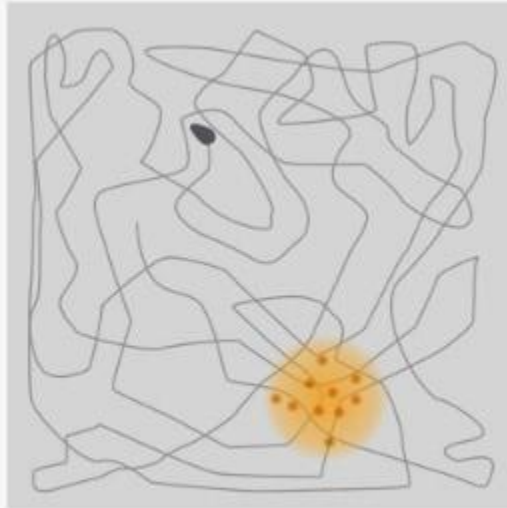




Nobelpreis für Medizin und Physiologie 2014



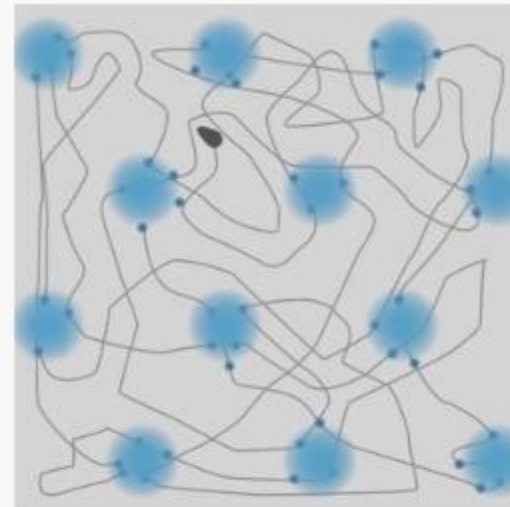
John O'Keefe



May-Britt Moser



Edvard I. Moser



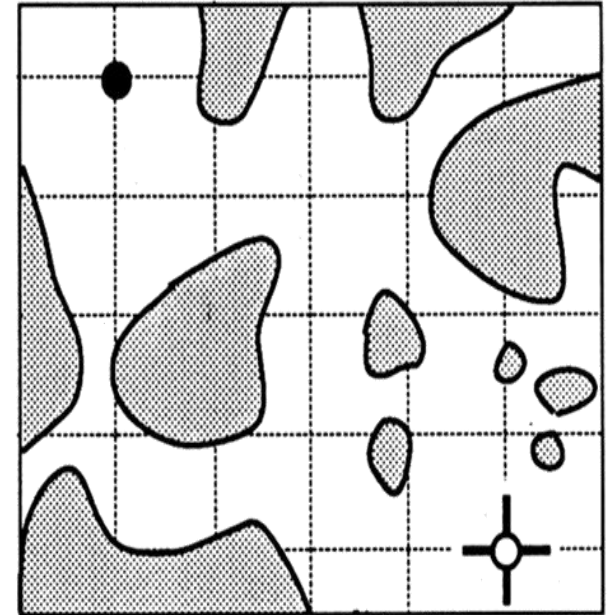
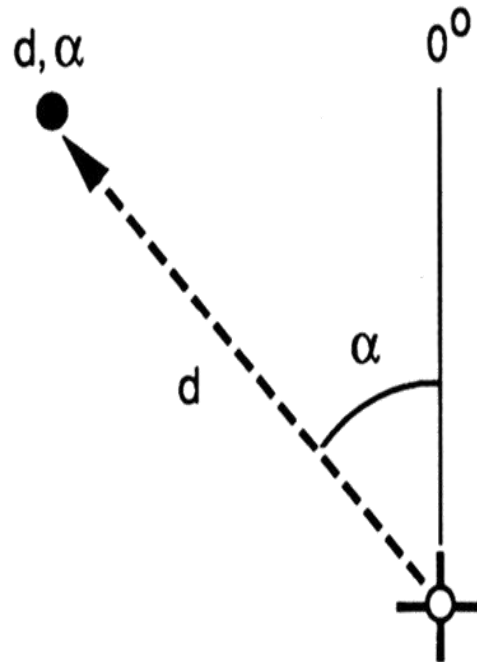
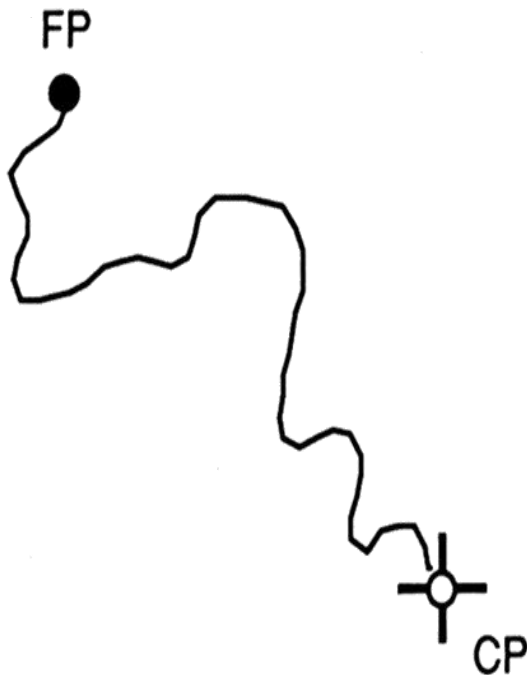
Grundsätzliche Navigationsstrategien

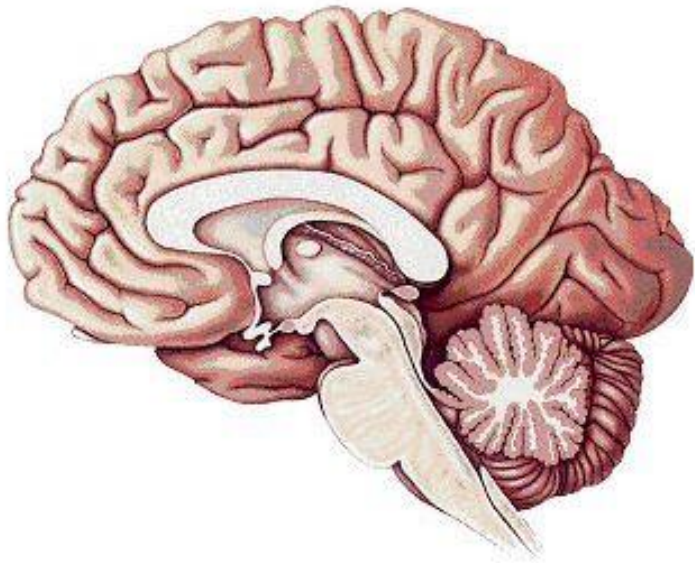
wegebasierte Systeme

kartenbasierte Systeme

dem Weg folgen

Wegintegration



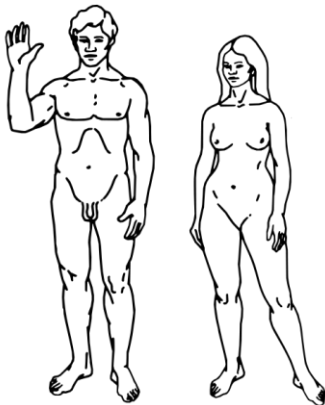


ca. 1,4 kg
 10^{11} Neuronen



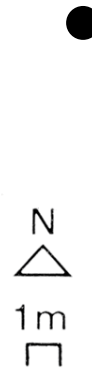
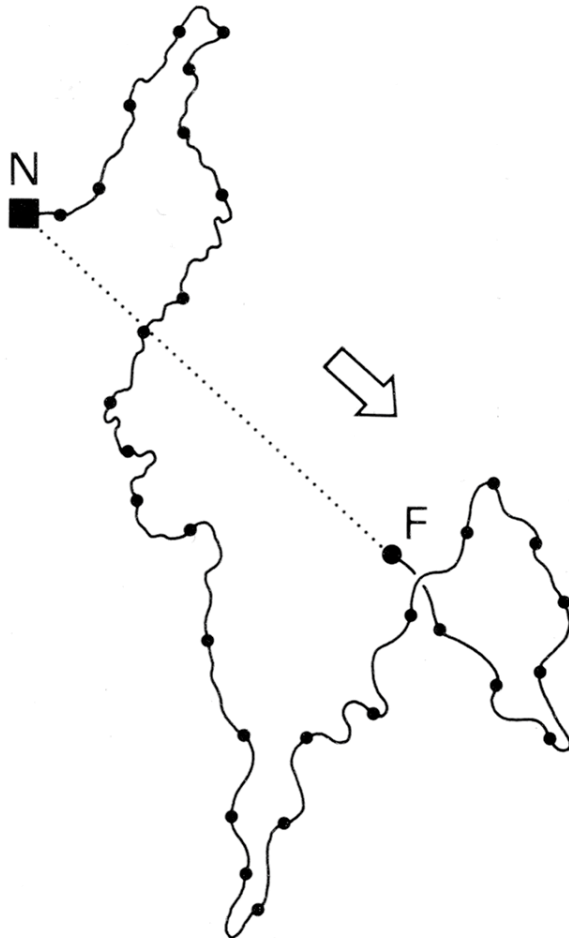
ca. 0,1 mg
 10^5 Neuronen

Wehner 2003



Versetzungsversuch

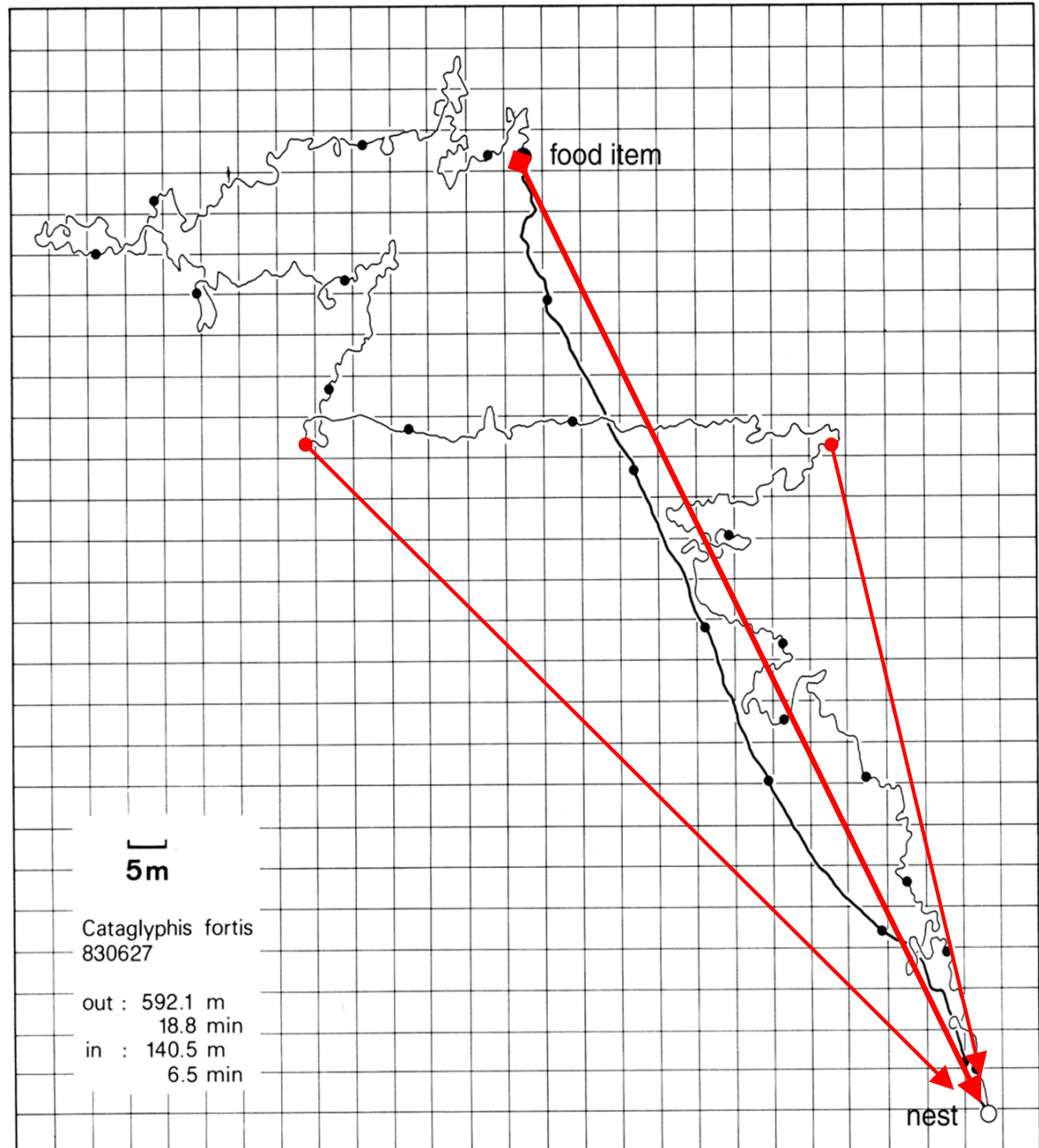
A



Wegintegration

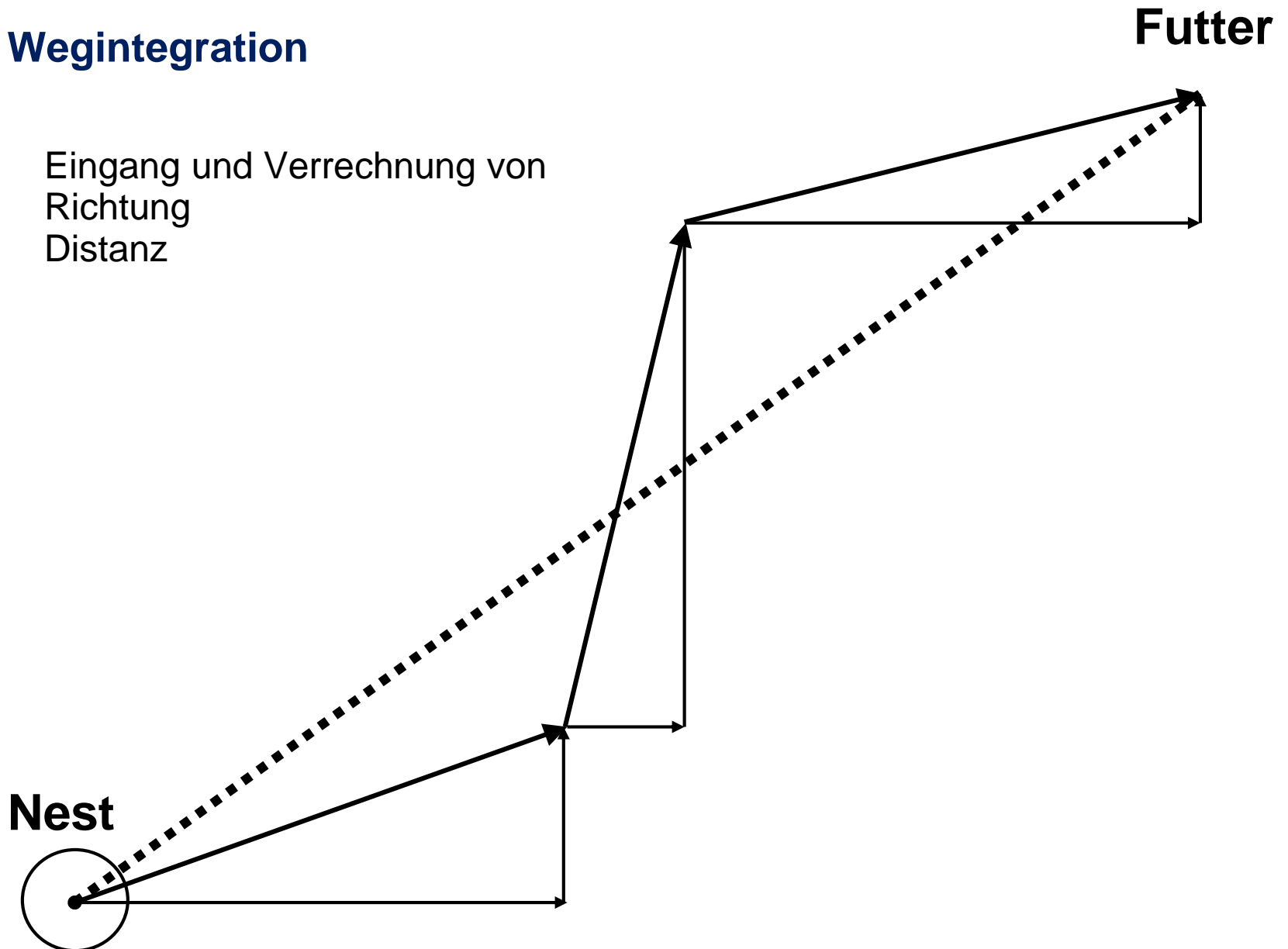
Schritt-für-Schritt
(angenäherte)
Vektoraddition

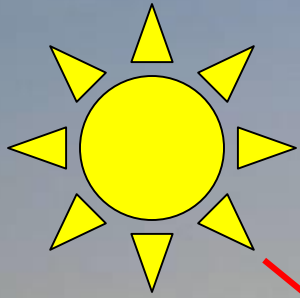
Suche
Ø 0.52 m/s
Rückweg
Ø 0.36 m/s



Wegintegration

Eingang und Verrechnung von
Richtung
Distanz



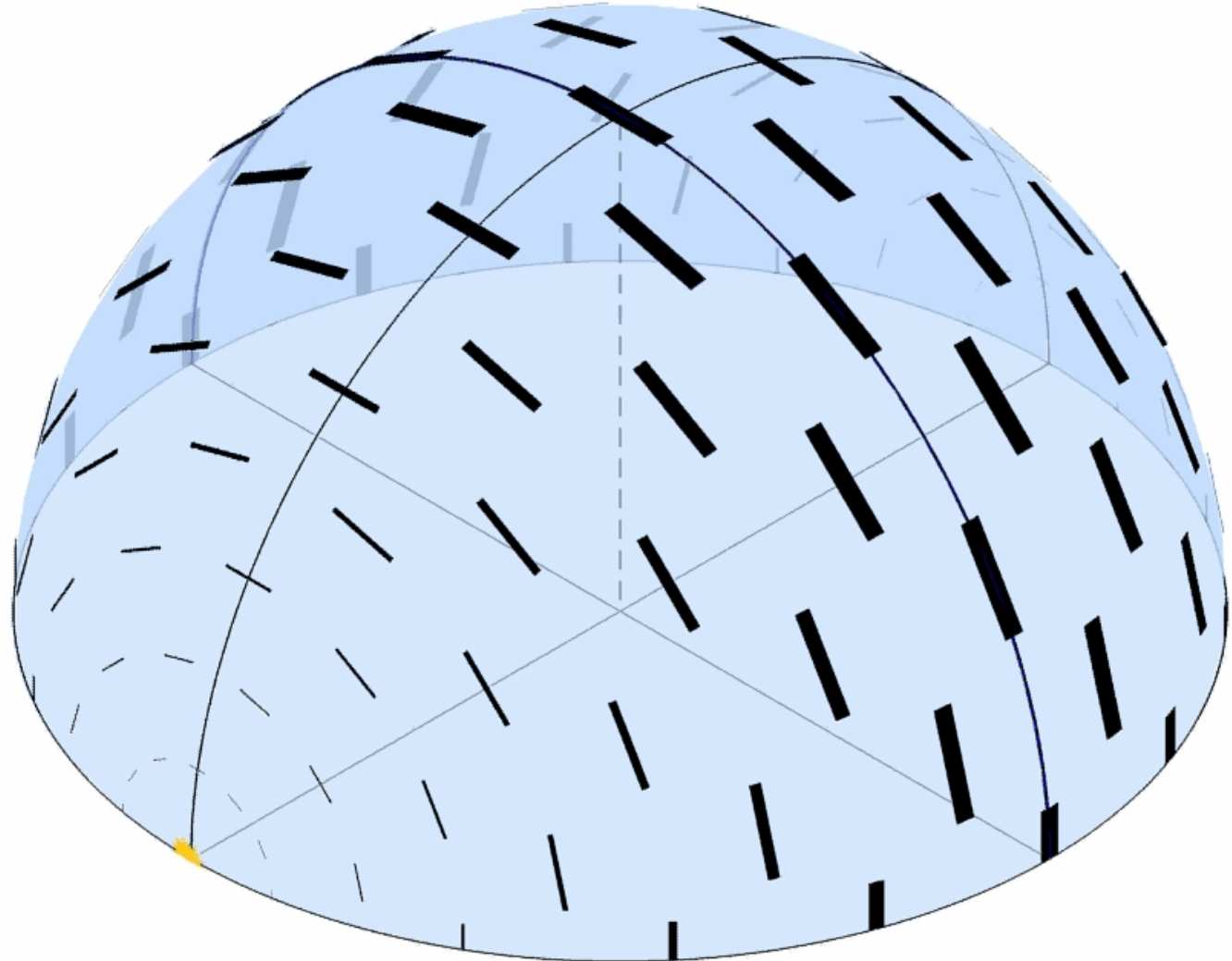


Wie wird die Richtung ermittelt?

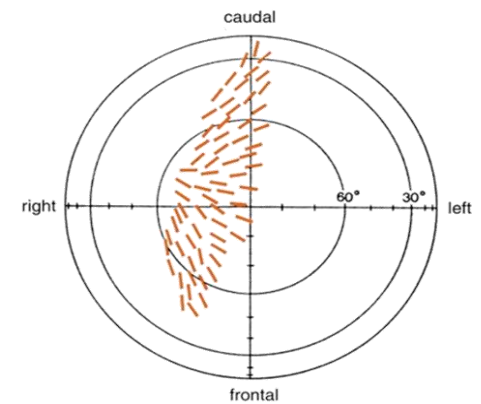
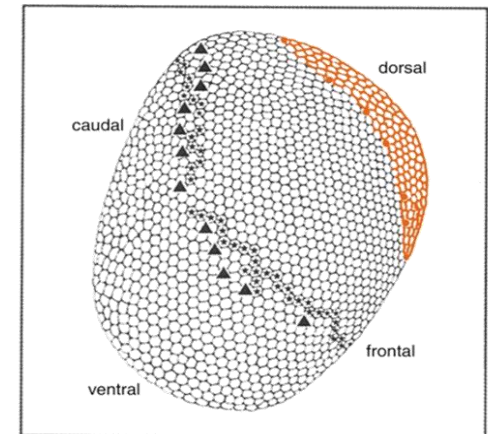
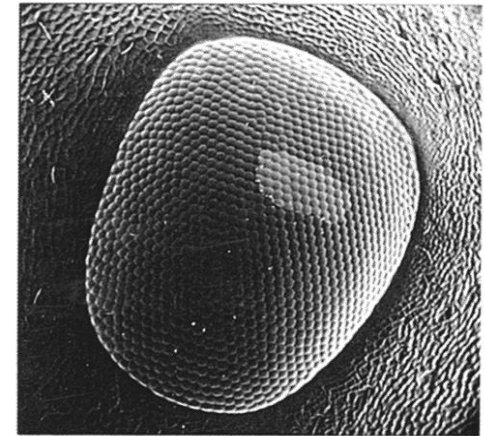
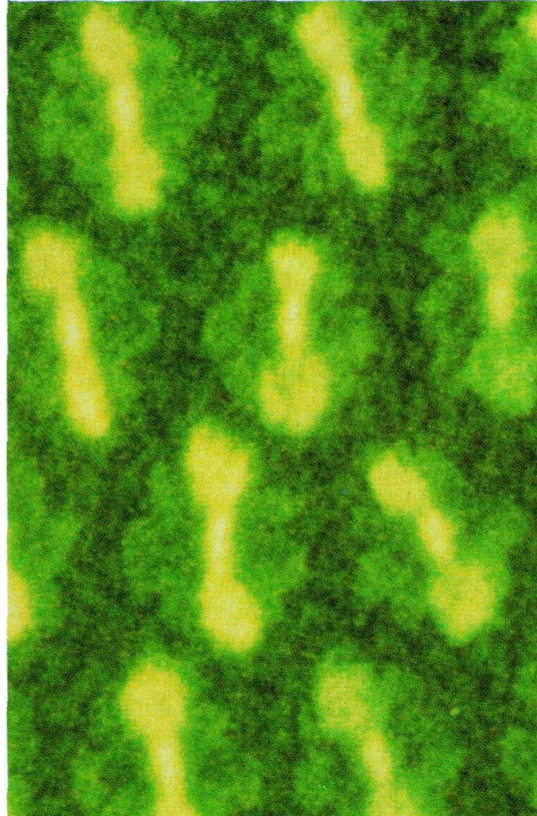
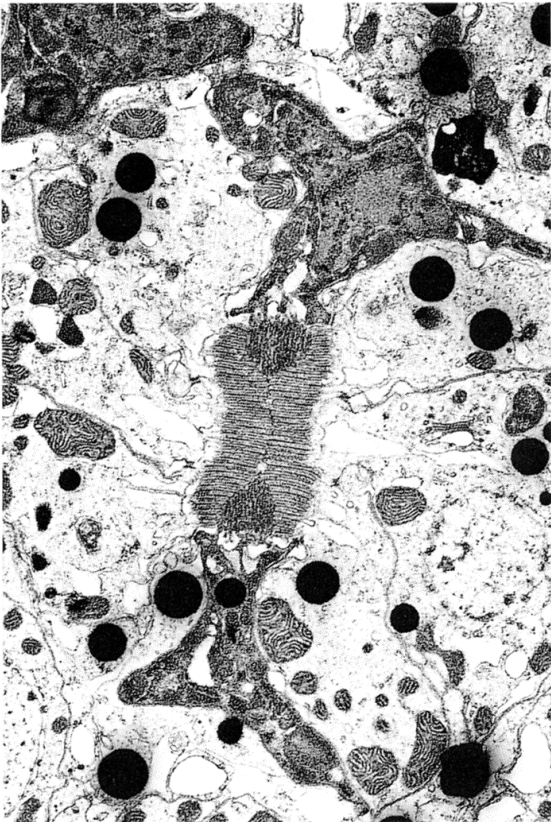
Nest



Sonnen- und Polarisationskompass



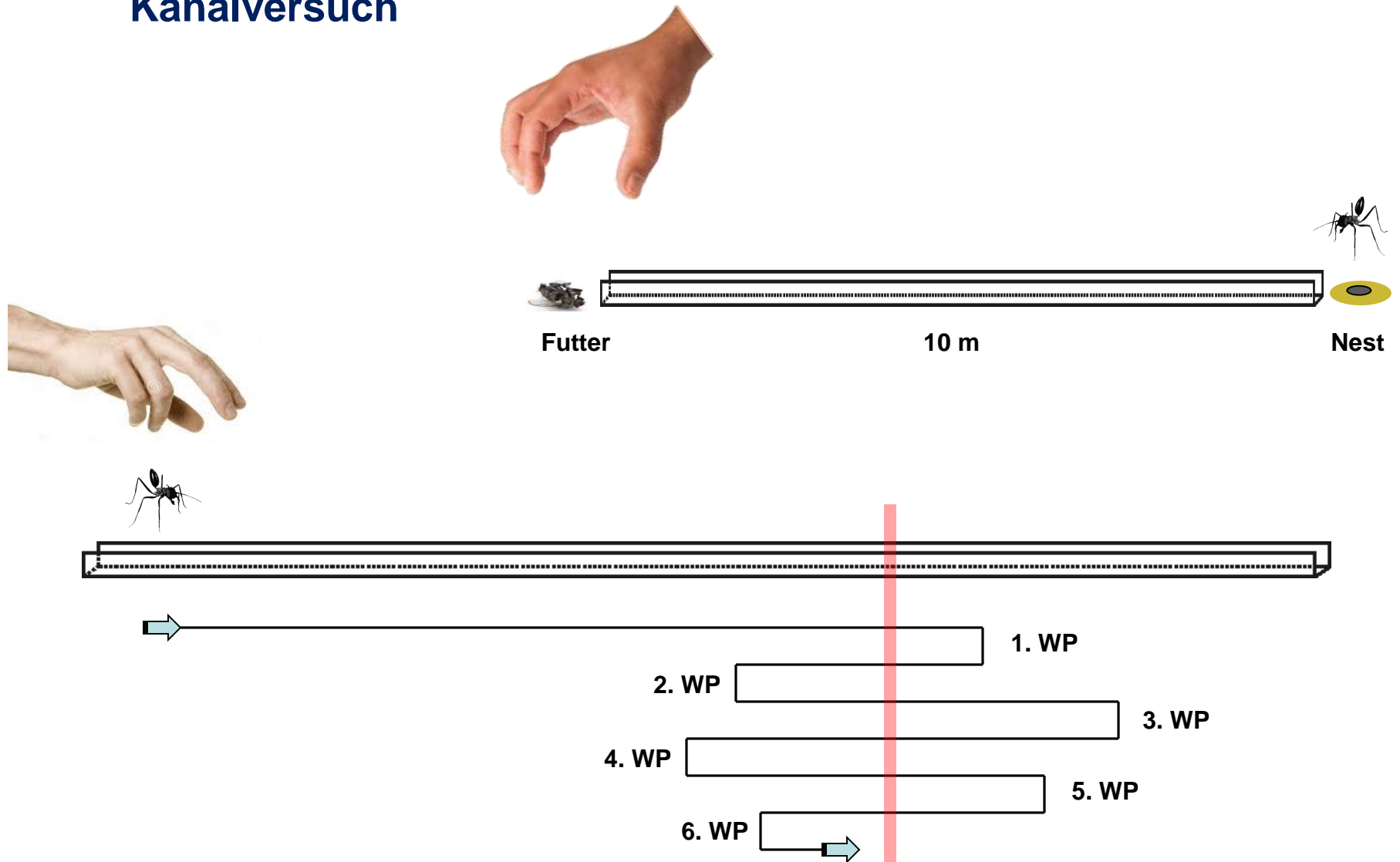
Filtereigenschaft der Ommatidien dorsalen Augenrand

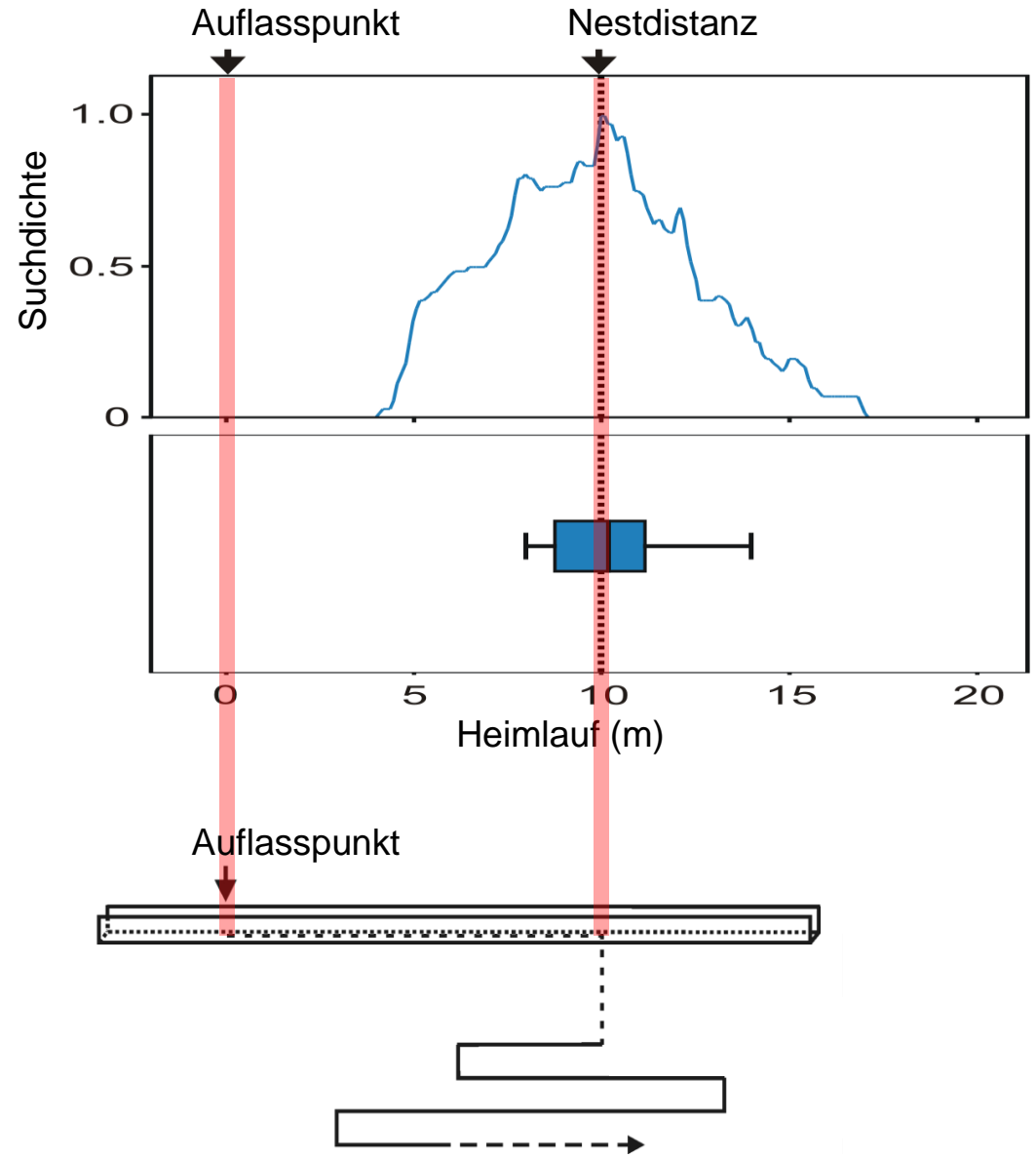
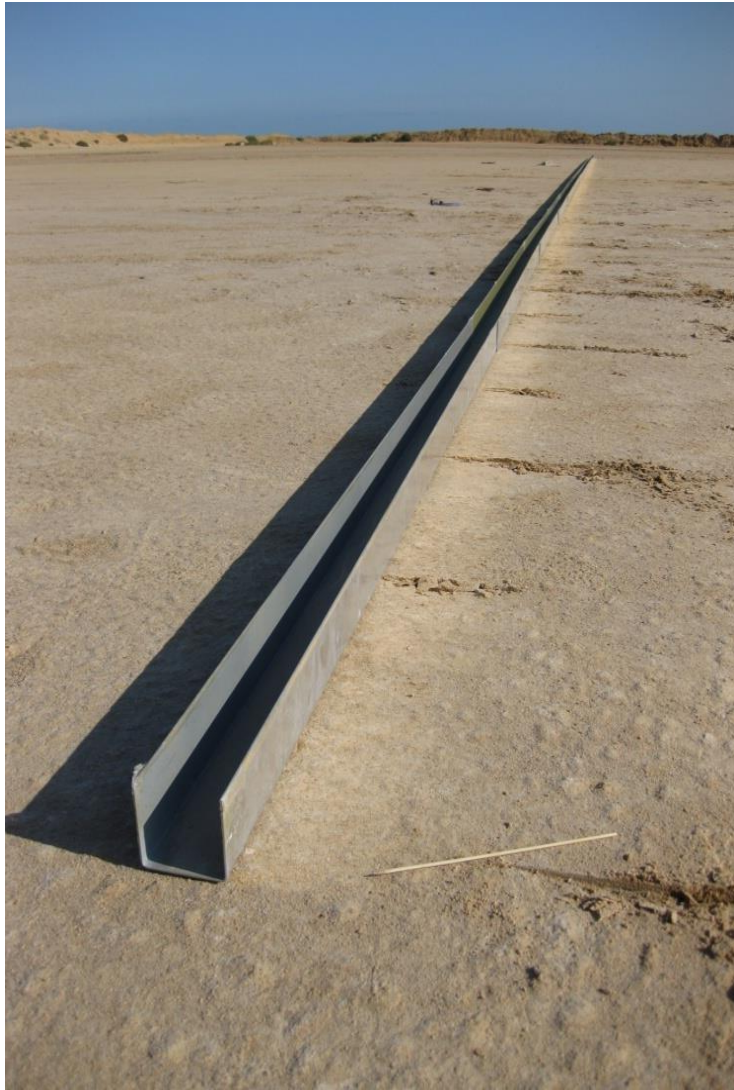


Distanzmessung Kanalversuch

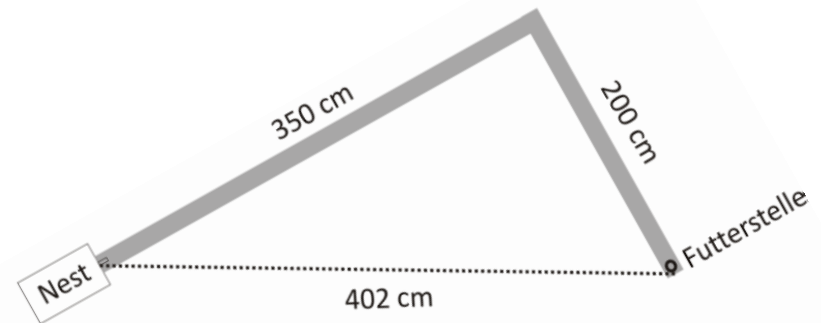
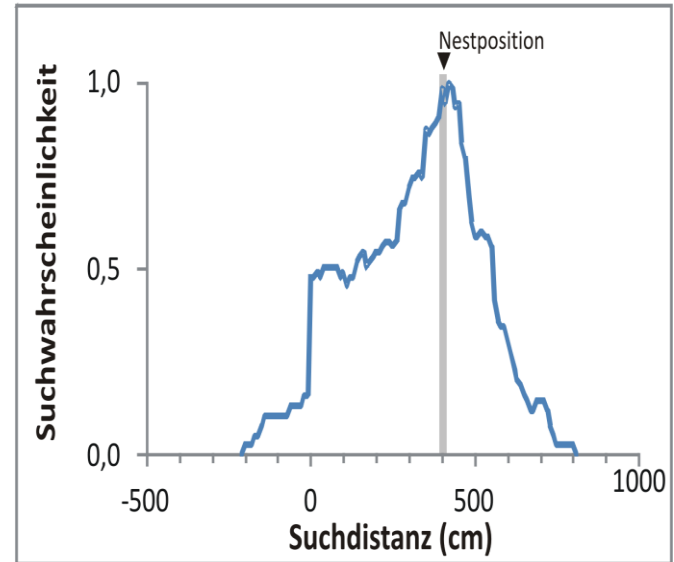


Distanzmessung Kanalversuch





Wegintegration Science Camp Sommer 2013



Testkanal

Temperaturen in den Kanälen (14-16 Uhr):
39,3 - 48,0 °C

Wie wird die Distanz ermittelt?

Energieverbrauch ?

Schäfer & Wehner 1993; Wohlgemuth et al. 2002



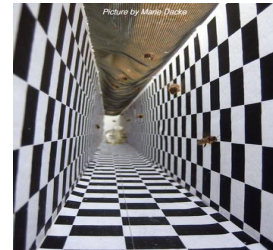
Zeitmesser ?

Wohlgemuth et al. 2001; 2002



Optischer Fluss ?

Ronacher & Wehner 1995; Ronacher et al. 2000



Schrittintegration ?

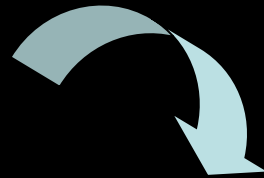
Wittlinger et al. 2006; 2007



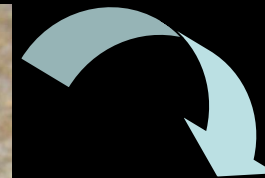
Beinverlust unterwegs



Aggression



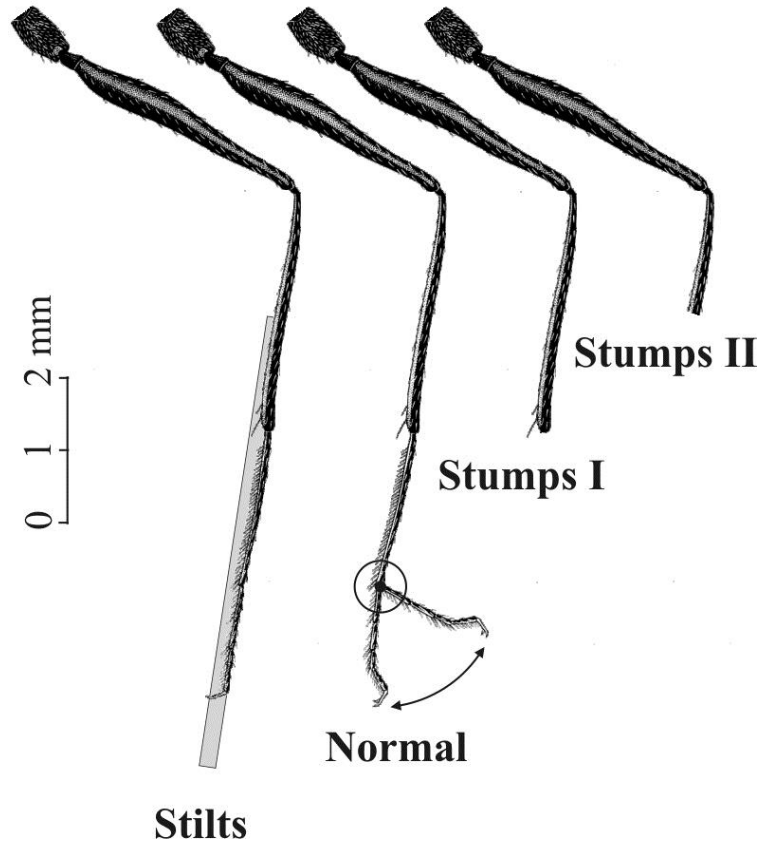
Kampf



“Verlierer”

Verlust: 1,5 Beine, 1 Antenne

Systematische Beinlängenänderung



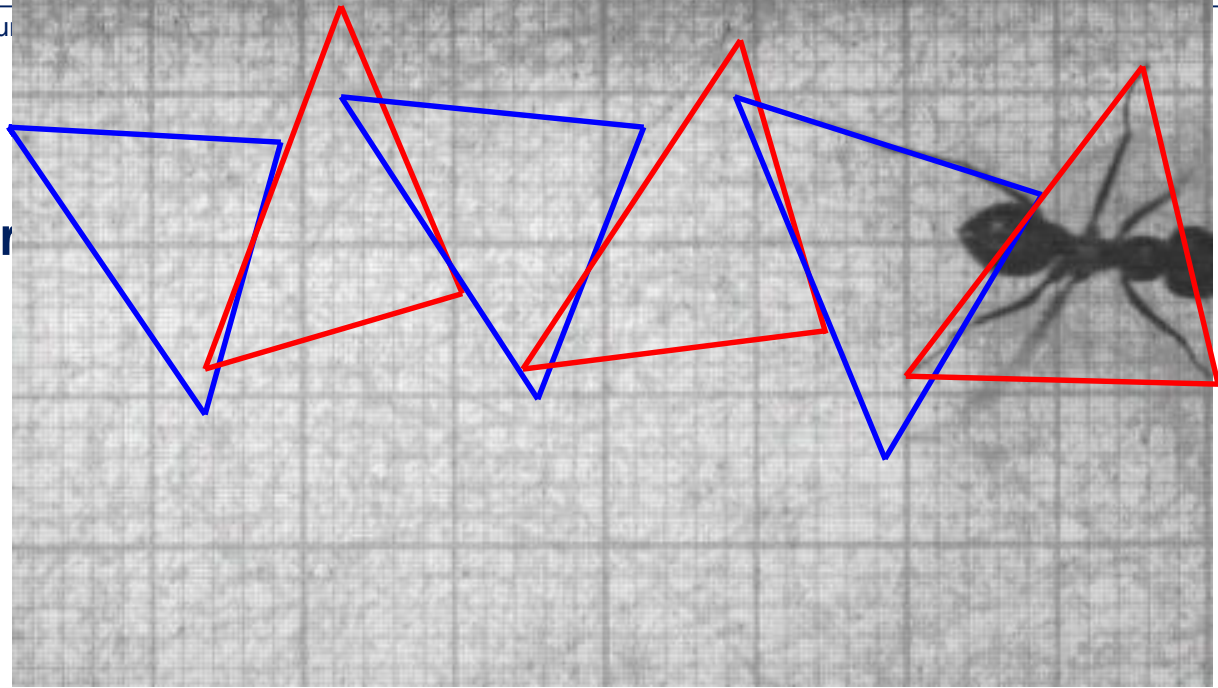




normal

$v = 0,28 \text{ m/s}$

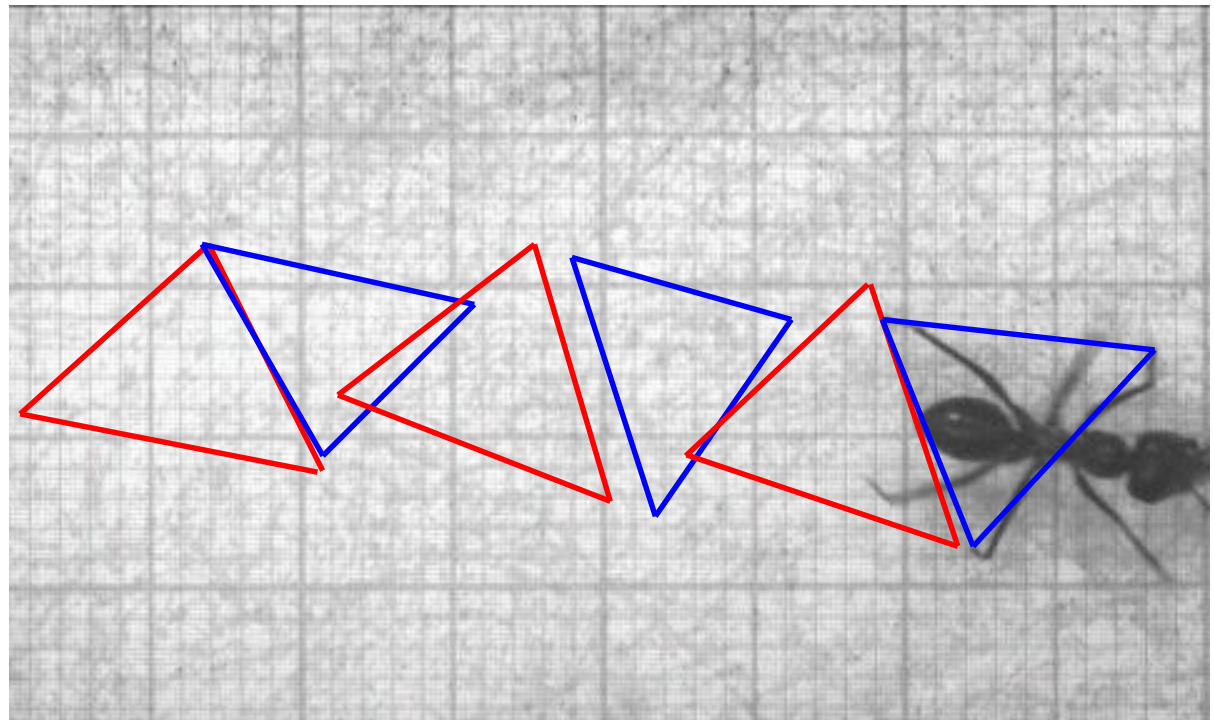
$sl = 14 \text{ mm}$

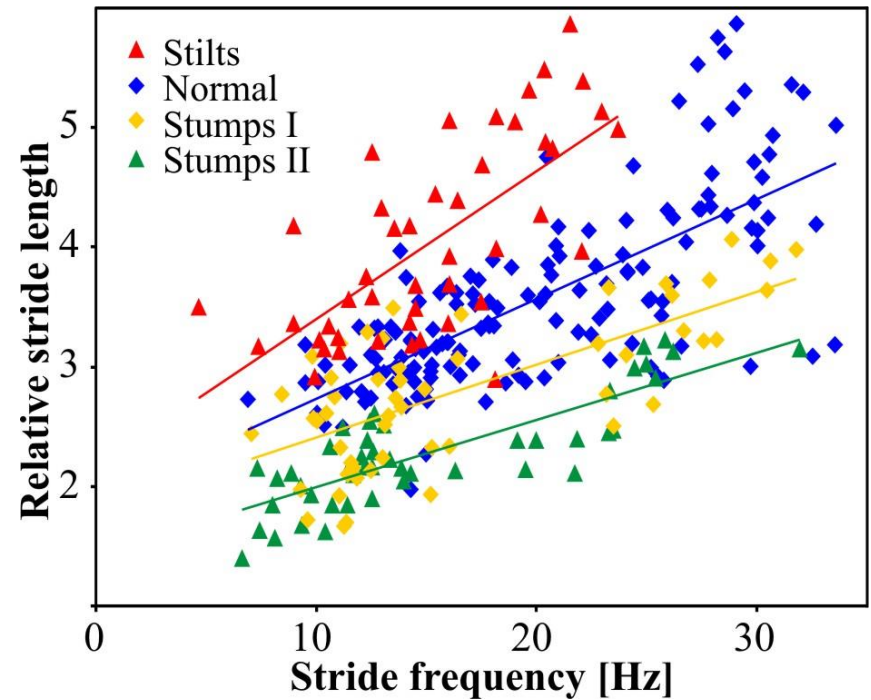
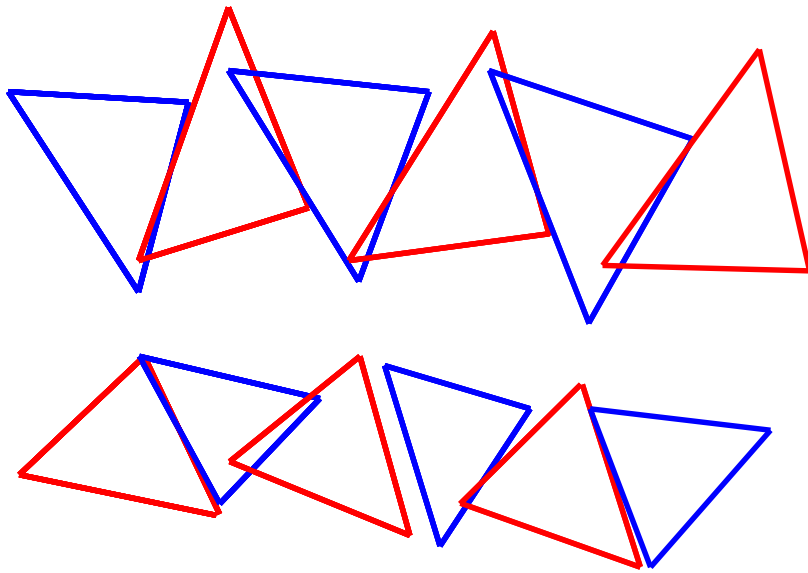


stumps II

$v = 0,27 \text{ m/s}$

$sl = 11 \text{ mm}$



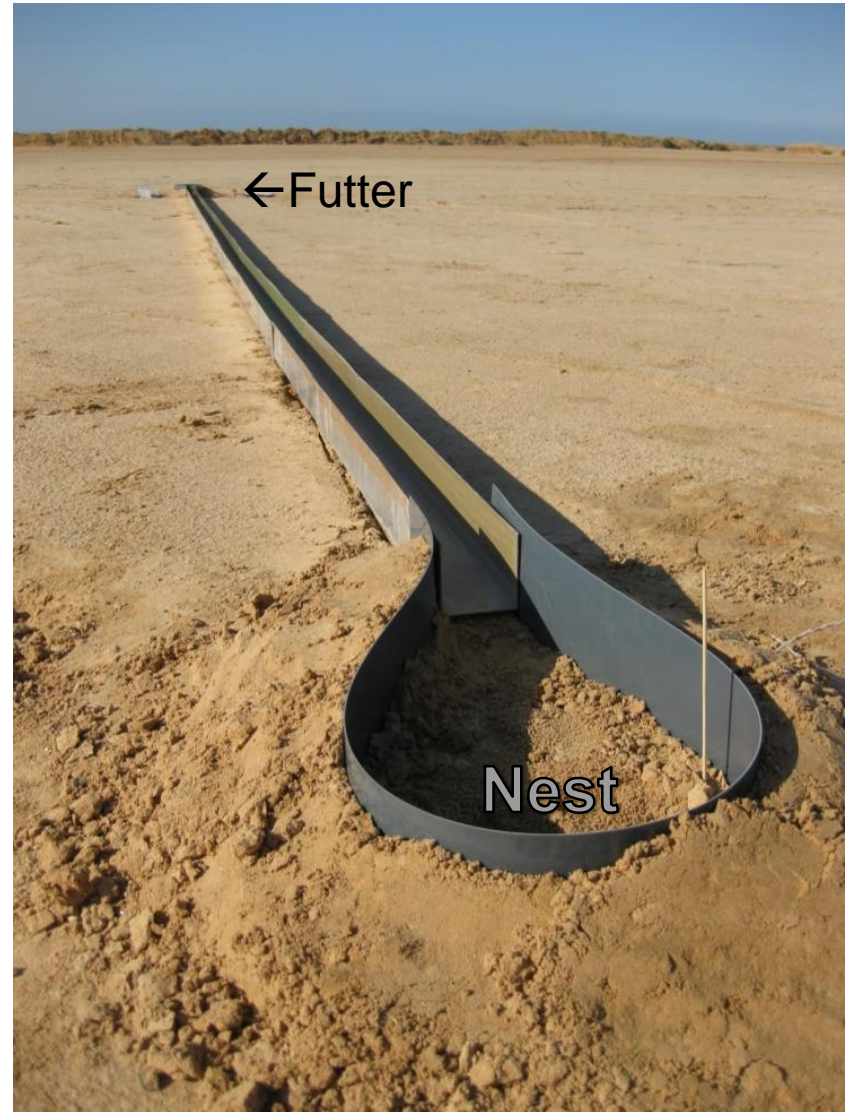


Tripode Gangart bleibt unverändert

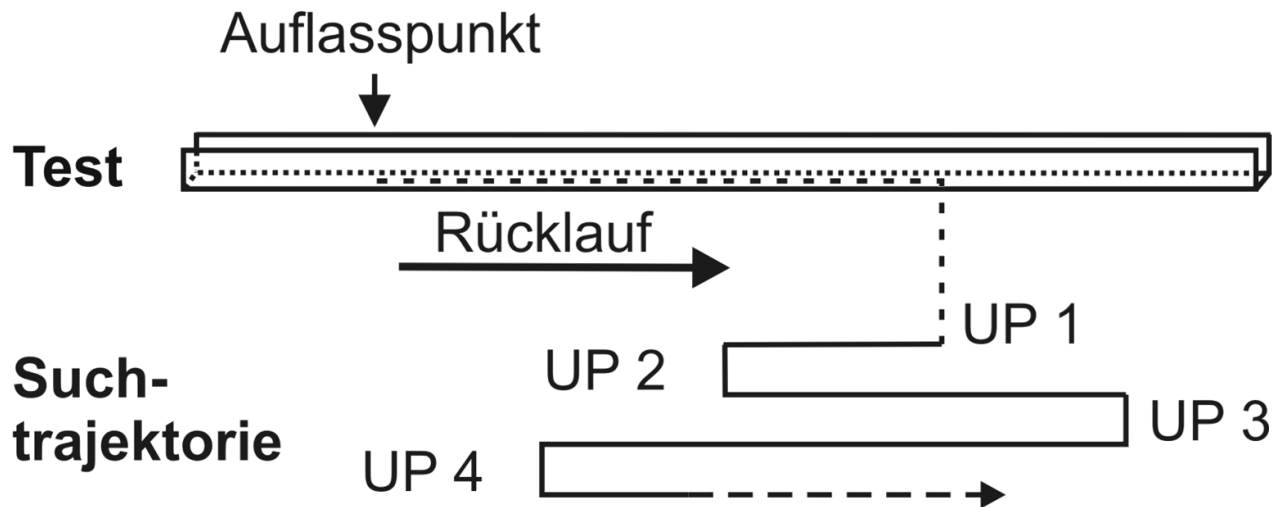
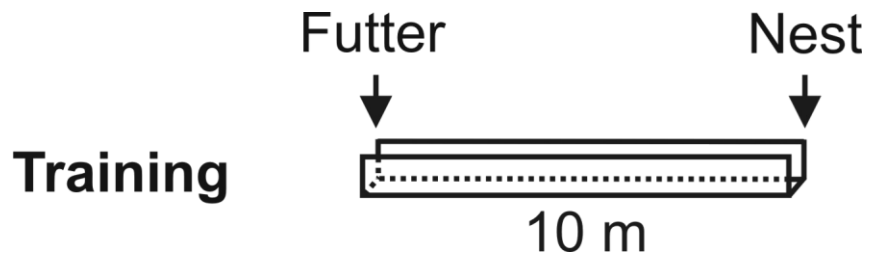
Veränderung der Schrittlängen

- kurze Beine – kurze Schritte
- lange Beine – lange Schritte

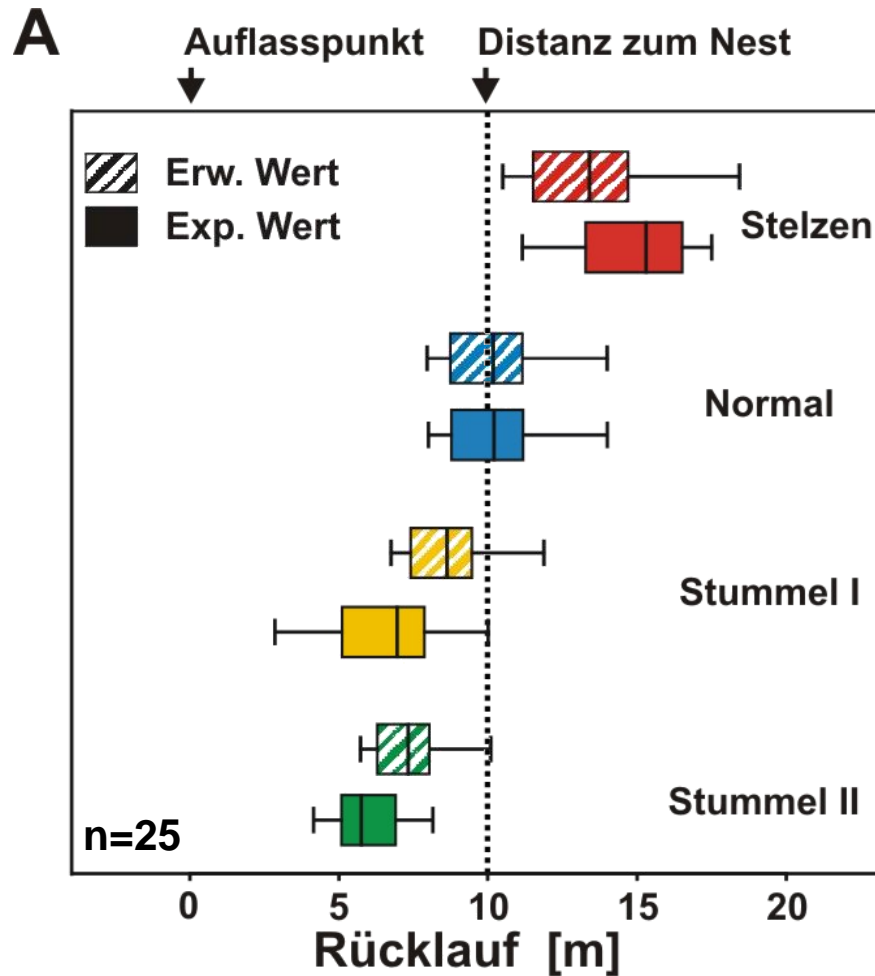
Beziehung zwischen Frequenz und Schrittlänge bleibt erhalten



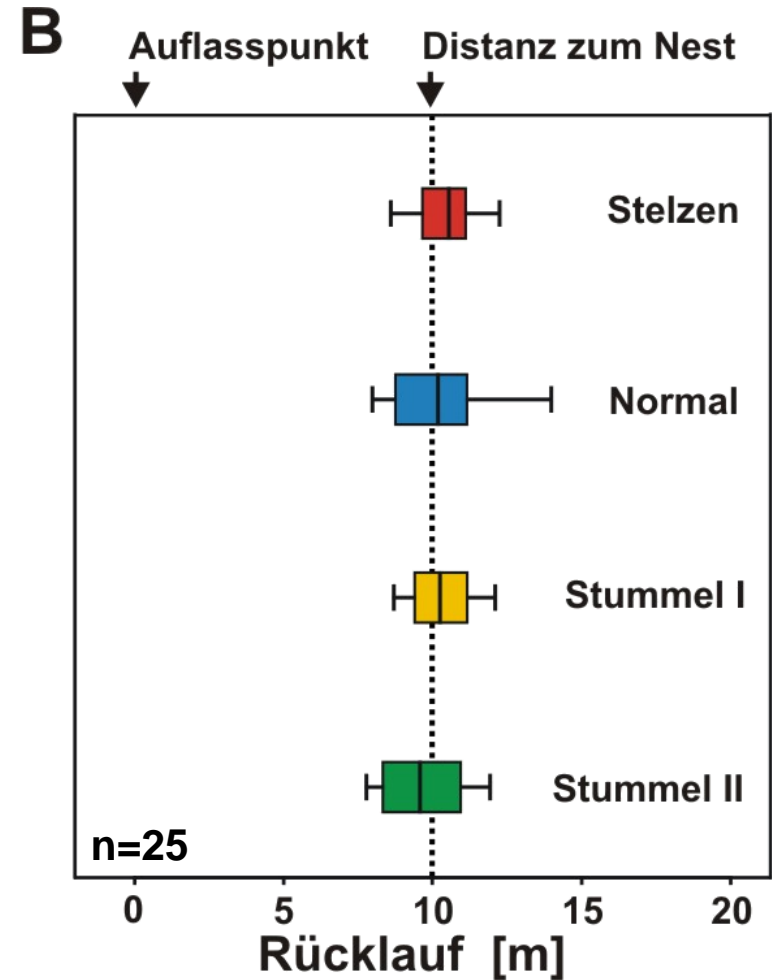
Odometer



Hinlauf - Rücklauf
normal manipuliert



Hinlauf - Rücklauf
manipuliert manipuliert

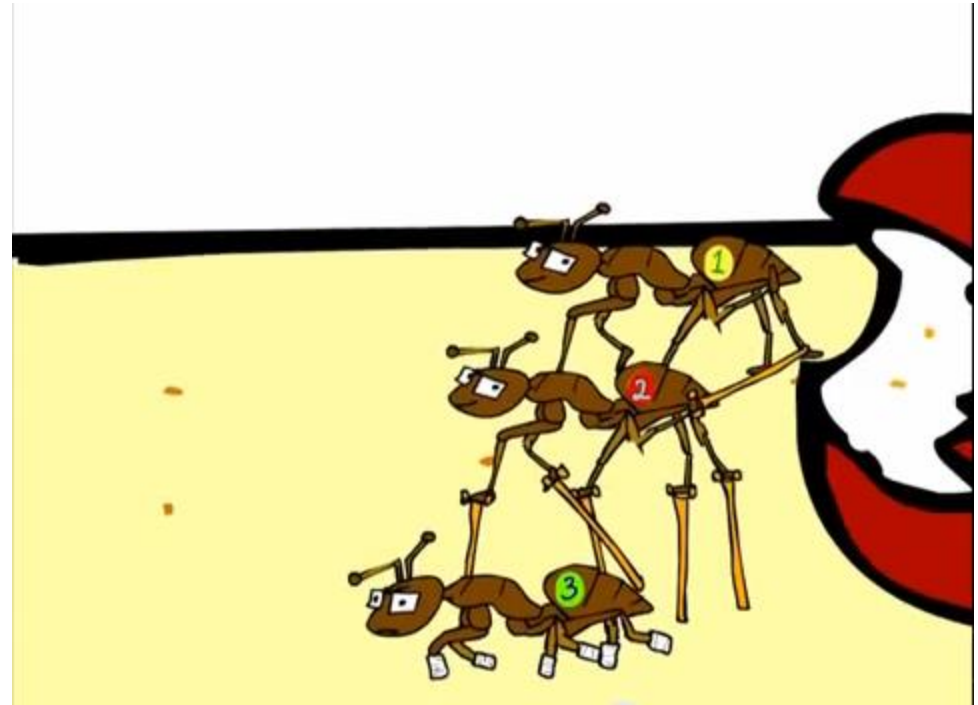


Cataglyphis Wüstennameisen

Wegintegration

Richtung:
Himmelskompass

Entfernung:
Schrittintegration



<http://www.youtube.com/watch?v=7DDF8WZFnoU>



Wie wird die Distanz ermittelt?

Energieverbrauch ?

Schäfer & Wehner 1993; Wohlgemuth et al. 2002



Zeitmesser ?

Wohlgemuth et al. 2001; 2002



Optischer Fluss ?

Ronacher & Wehner 1995; Ronacher et al. 2000

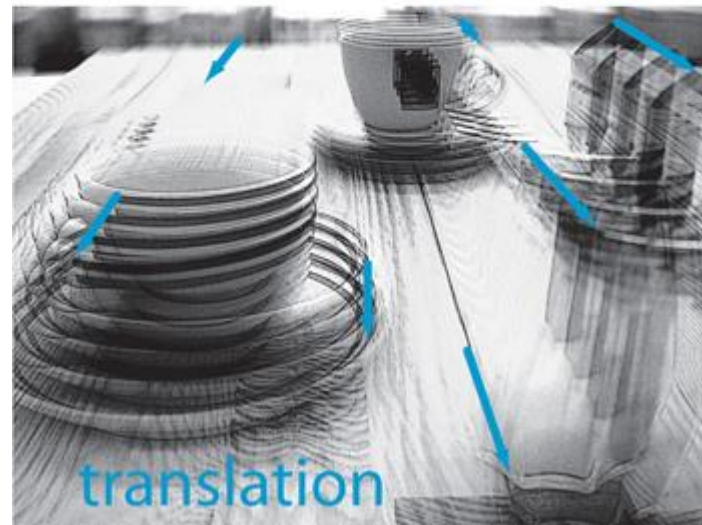
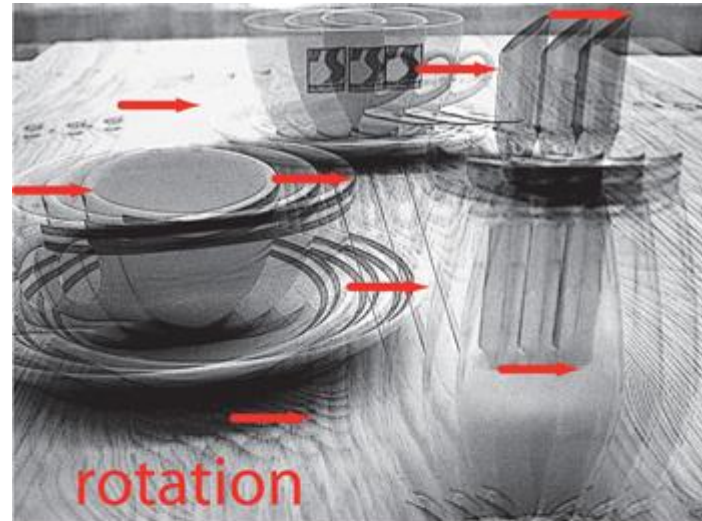


Schrittintegration !

Wittlinger et al. 2006; 2007



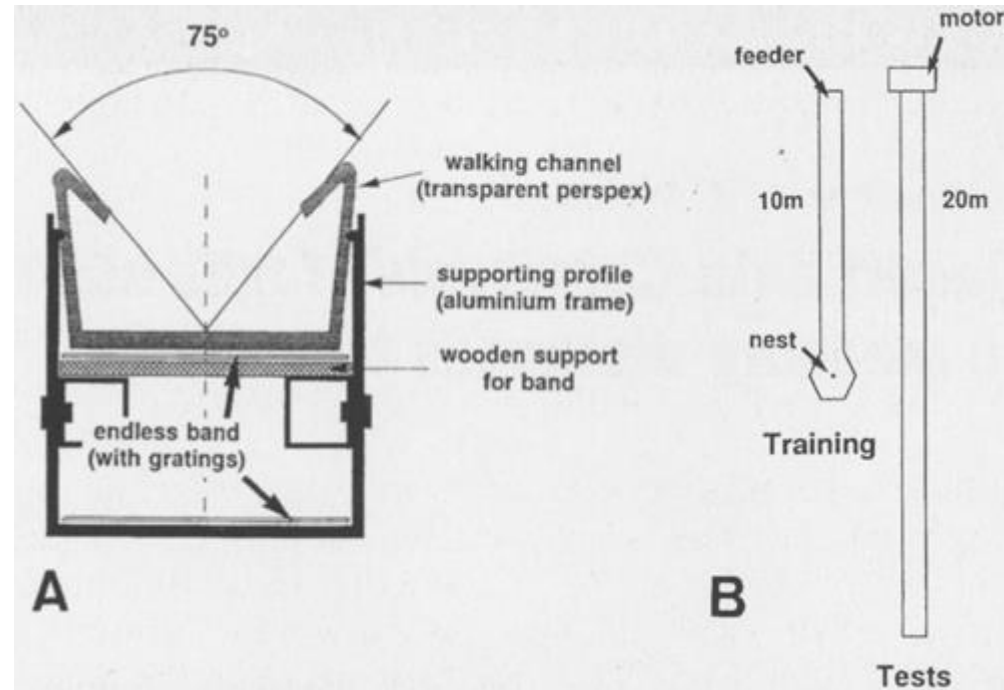
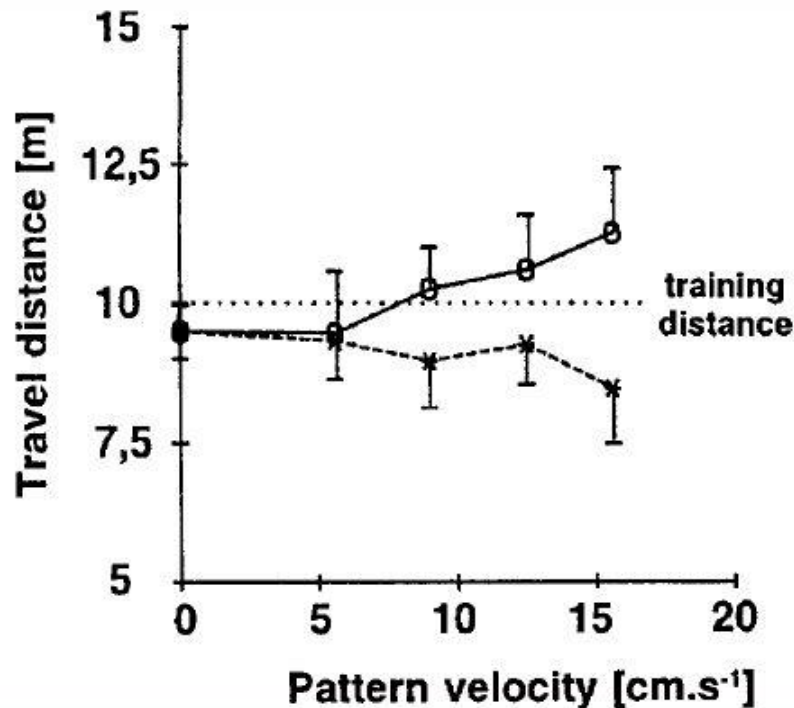
Was ist optischer Fluss?



ORIGINAL PAPER

B. Ronacher · R. Wehner

Desert ants *Cataglyphis fortis* use self-induced optic flow to measure distances travelled



Ameisen laufen und sehen

Verhaltensexperiment gesucht bei dem die beiden Inputs klar voneinander getrennt werden können.

Blinde Ameisen ???

=> zeigen, dass der Pedometer alleine ausreicht

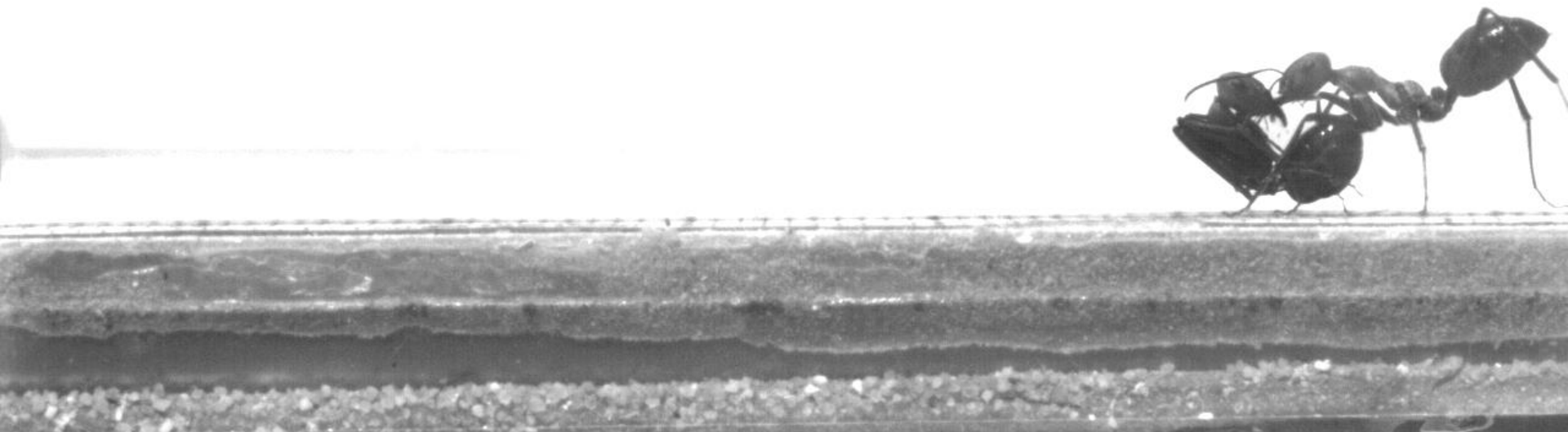


Nicht-laufende Ameisen ???



Carrier – Carried Paare

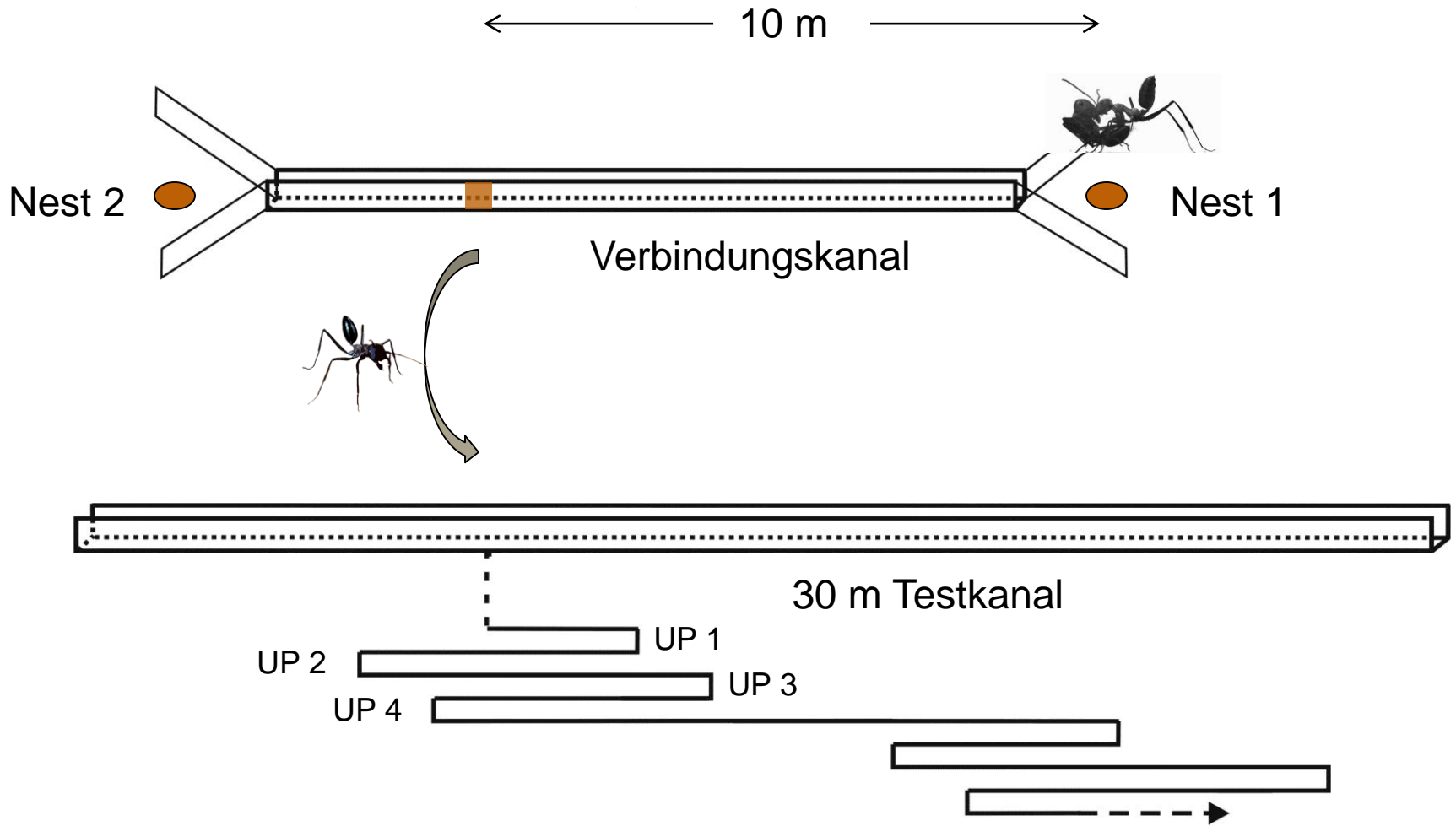
- Trägerin und Getragene Ameise erhalten denselben Betrag an Optischem Fluss
- Nur die Trägerin macht aktiv Lokomotion



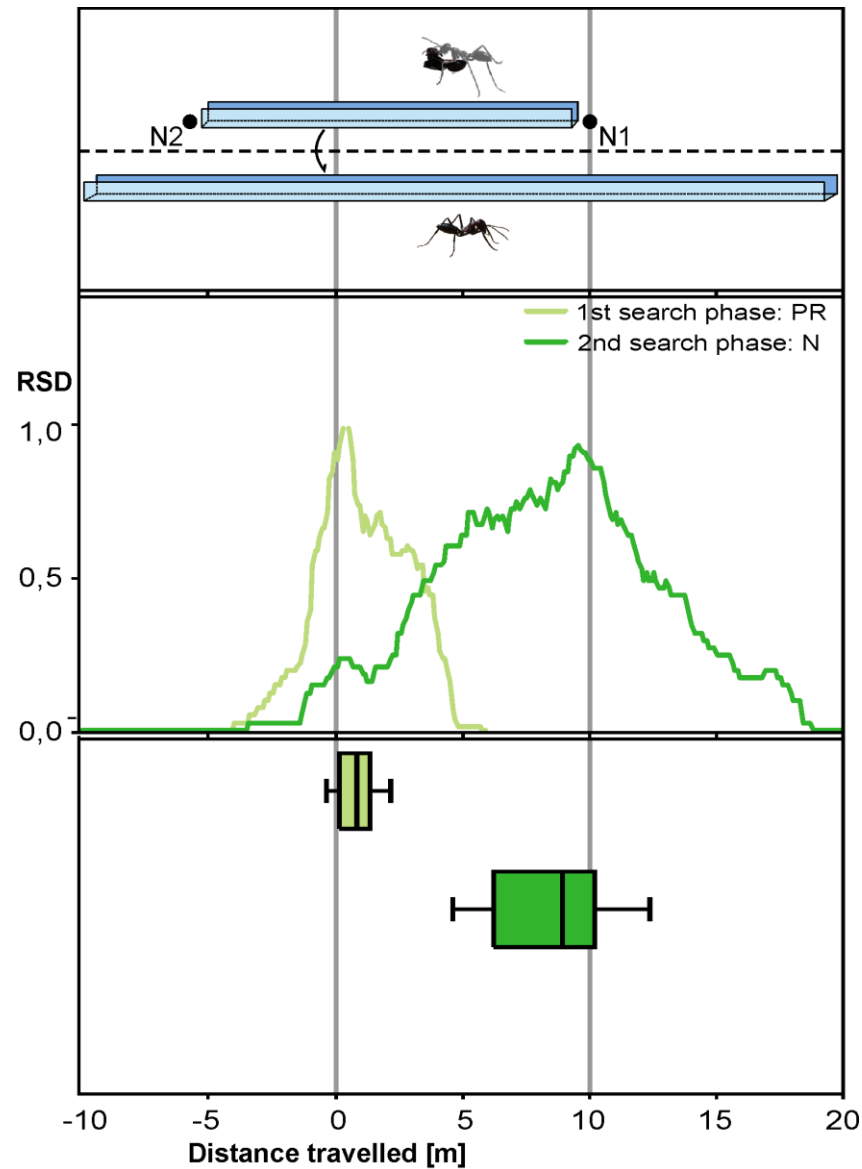


Nest 2 ●

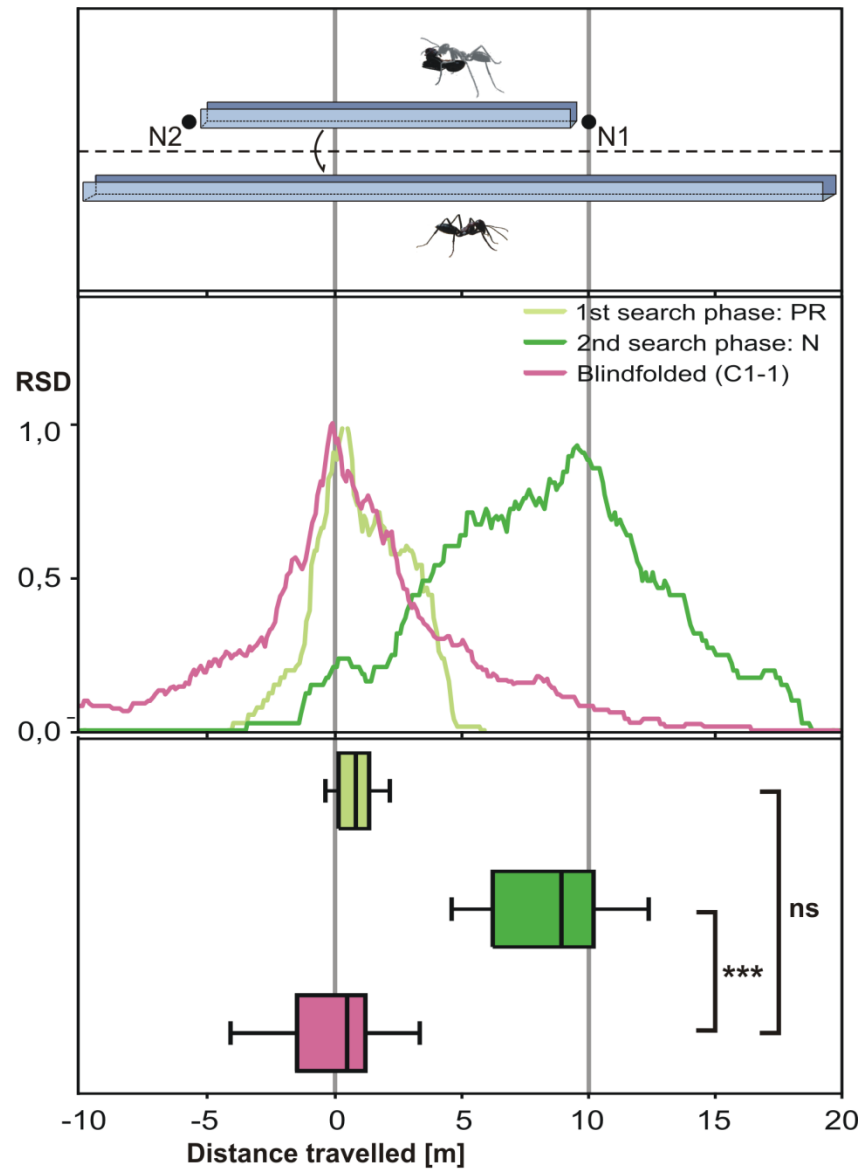
● Nest 1



Optischer Fluss - Experiment



Optischer Fluss - Experiment



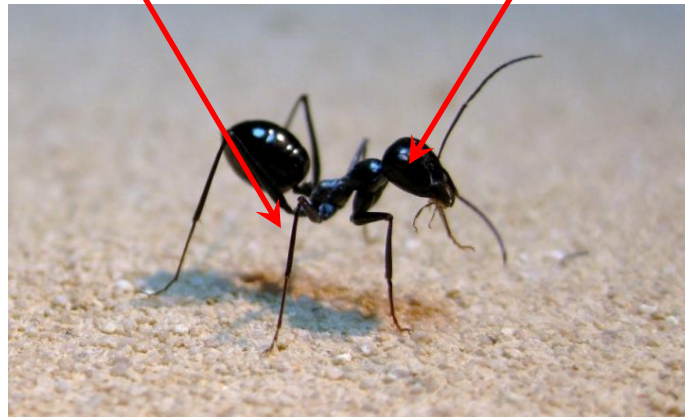
Odometry in Cataglyphis



step integration

!

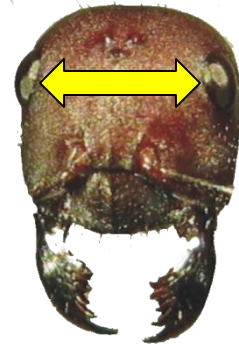
optic flow integration



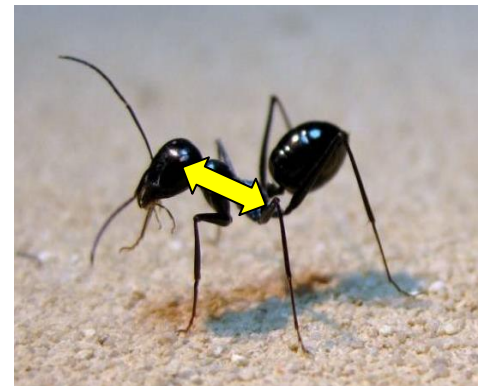
Questions

Gibt es **Informationstransfer** zwischen:

- **Beiden Augen** (Inter-Ocular Transfer - IOT) ?

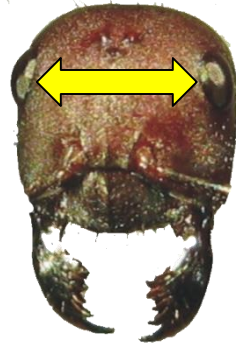


- **Beiden Odometersystemen** (OF-Meter - Pedometer) ?



Interocular transfer-experiment

- Interocular Transfer (IOT) of OF Information ist möglich



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LETTERS TO NATURE

NATURE VOL. 315 16 MAY 1985

Does interocular transfer occur in visual navigation by ants?

Rüdiger Wehner & Martin Müller

Department of Zoology, University of Zurich,
Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

If an animal that learns to respond to a visual stimulus presented to only one eye can continue to respond accurately when the stimulus is presented to the other eye, it is said to exhibit interocular transfer (IOT). IOT has been studied extensively in many groups of vertebrates¹⁻¹¹, but of IOT in an invertebrate (*Oc* an insect, the desert ant *Catagly* by the pattern of polarized li when it uses landmarks for ori behavioural performance ma strategies adopted by insects and terrestrial cues for naviga

Insects possess multi-facet them with full panoramic vis used the long-legged Saharan which the visual fields of the two eyes cover 93% of the unit

Wehner & Müller, 1985

Polarization Kompass: IOT möglich
Landmarken: kein IOT

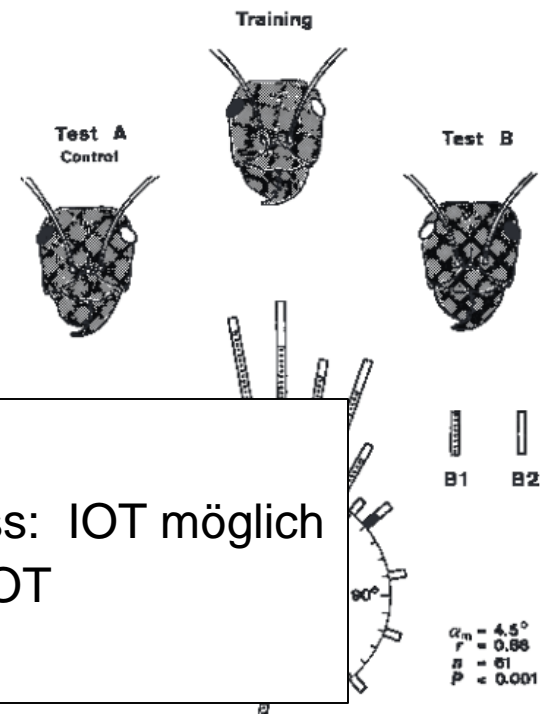
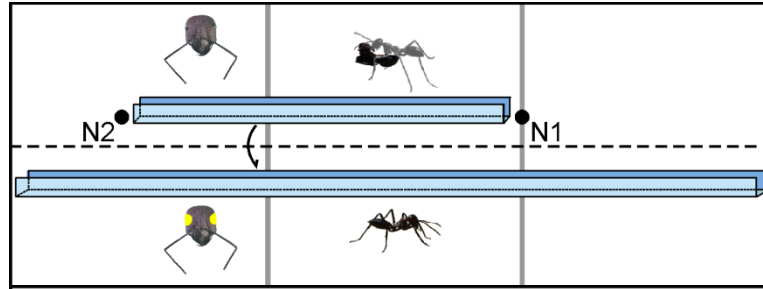
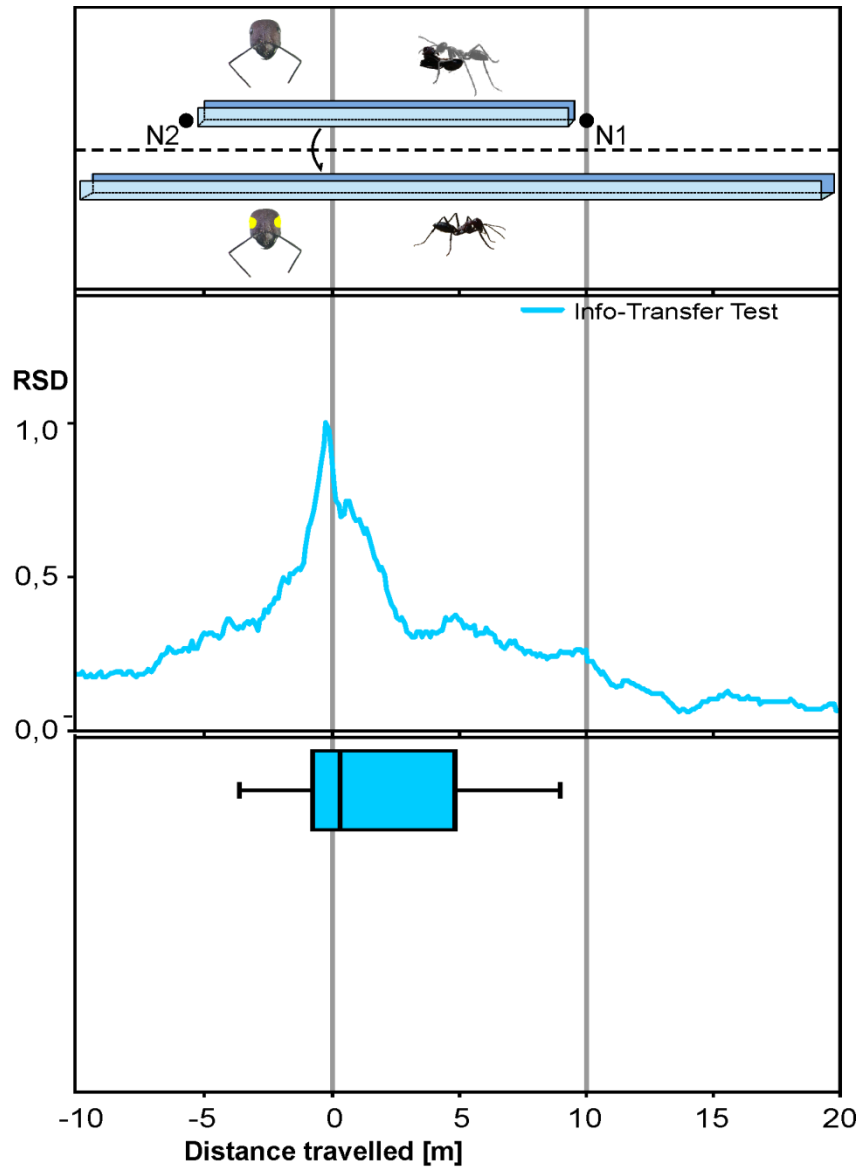


Fig. 1 Ants exhibit interocular transfer of visual information

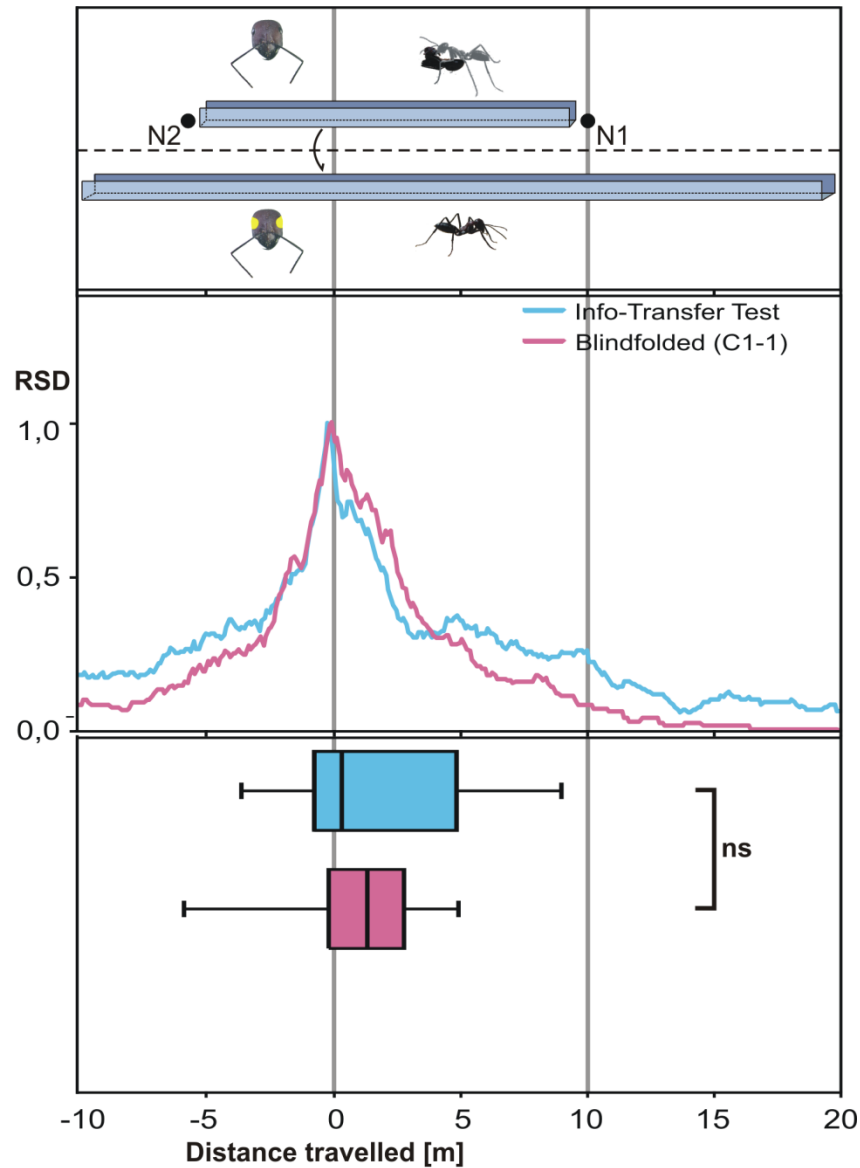
Odometer Information Transfer - Experiment



Odometer Information Transfer - Experiment



Odometer Information Transfer - Experiment



Odometer Informationstransfer- Experiment

- Kein Informationstransfer zwischen den Odometern

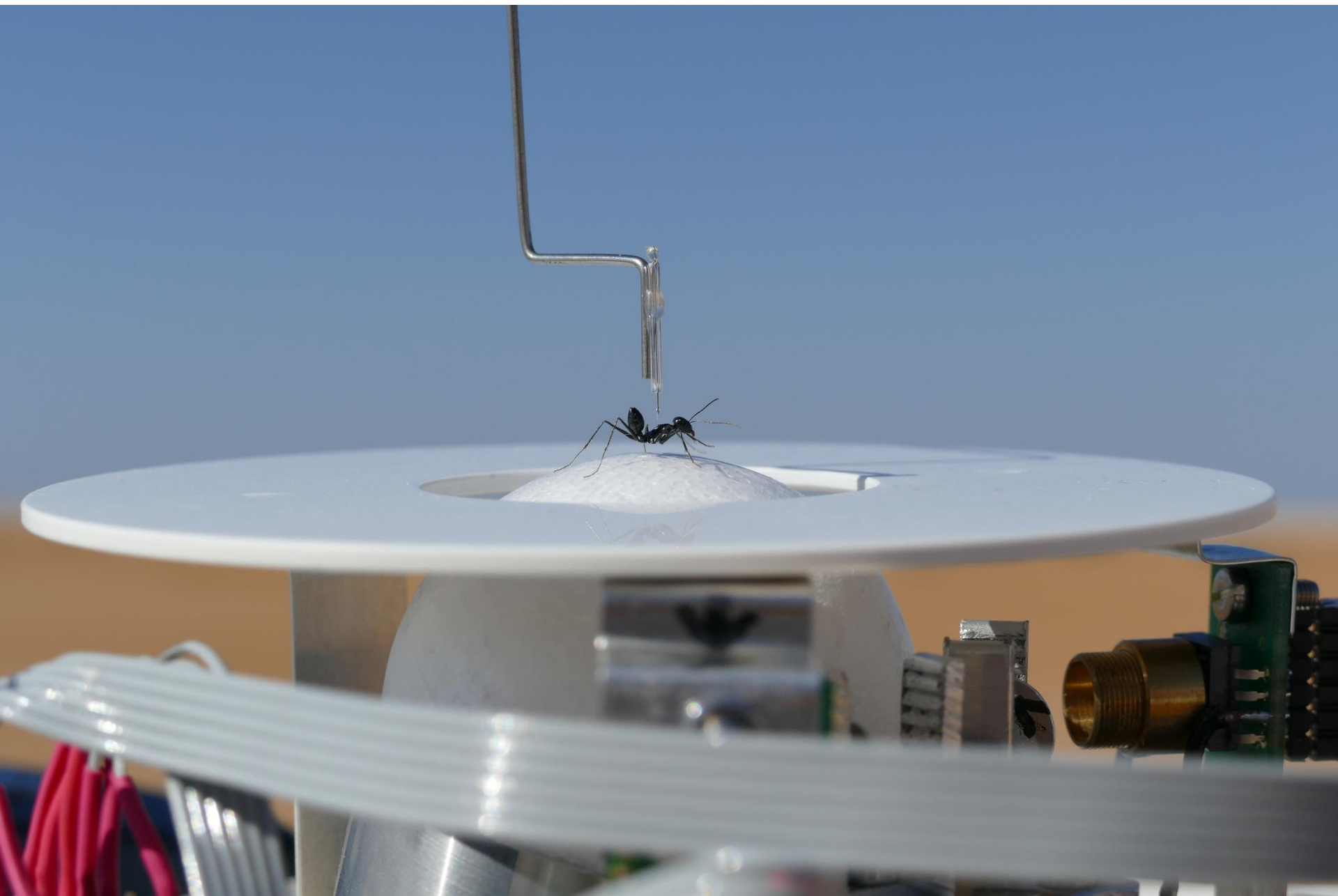


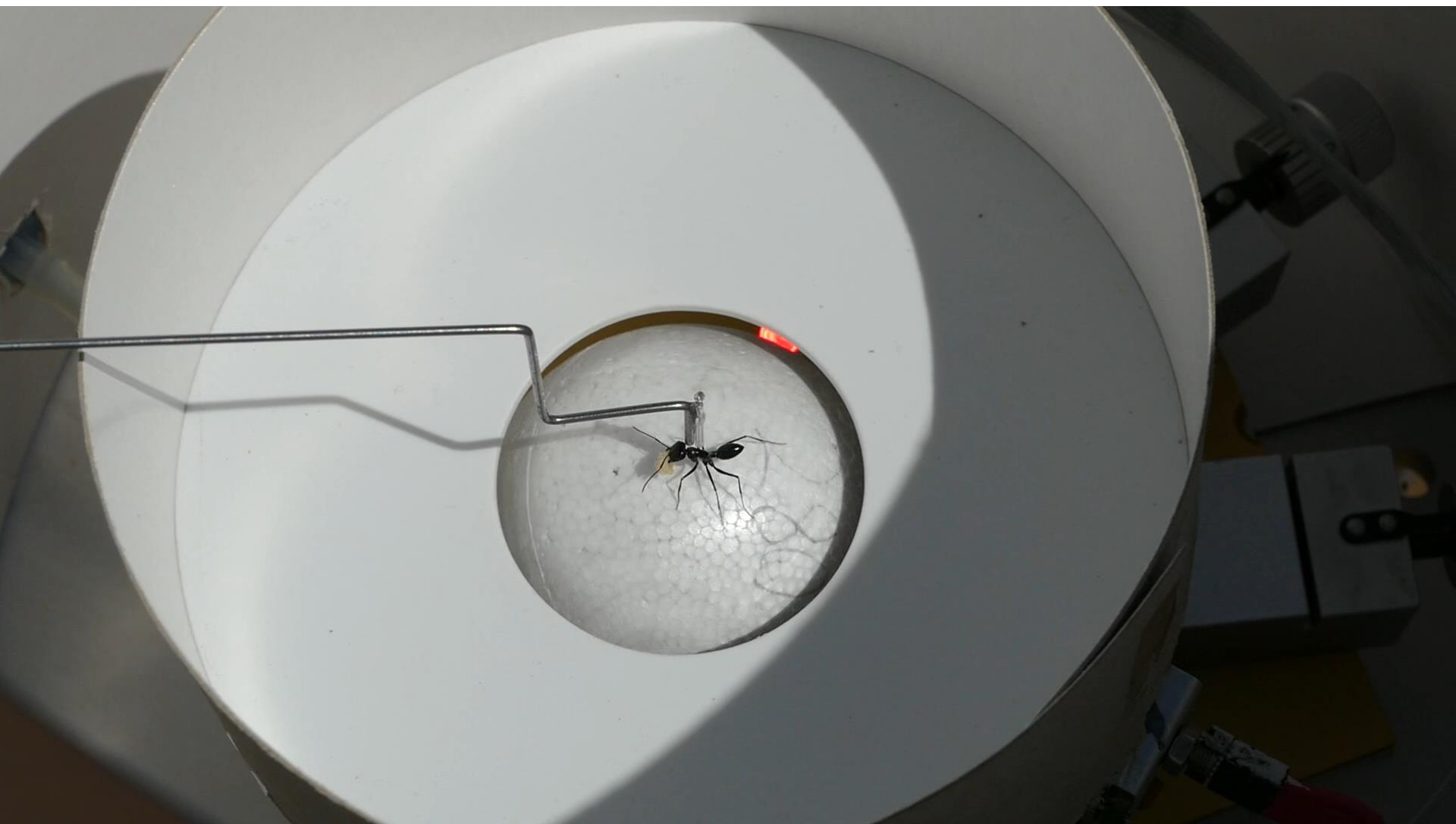
Cataglyphis Odometrie

- **Pedometer** – Entfernungsmessung durch **Schrittintegration**
- **Optischer ‘Flussmeter’** – Entfernungsmessung durch **OF-Integration**

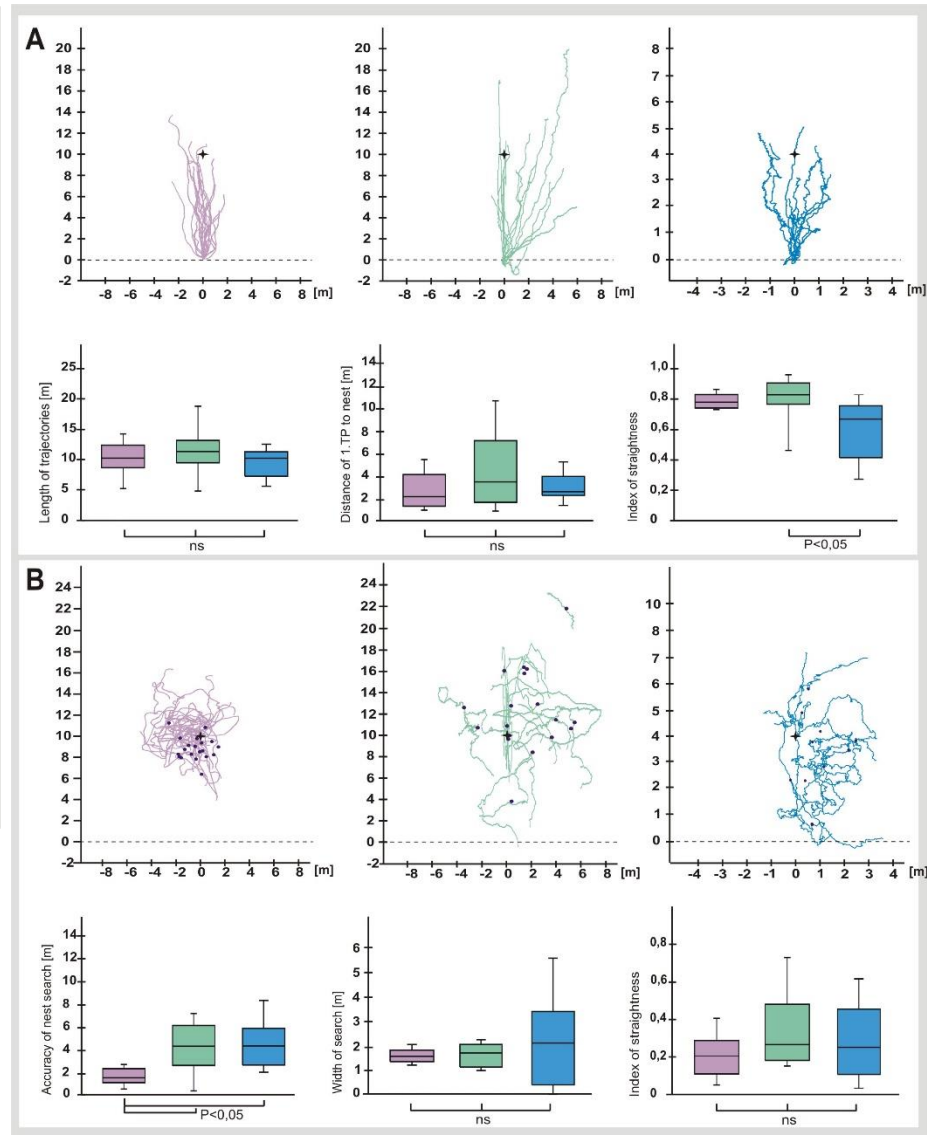
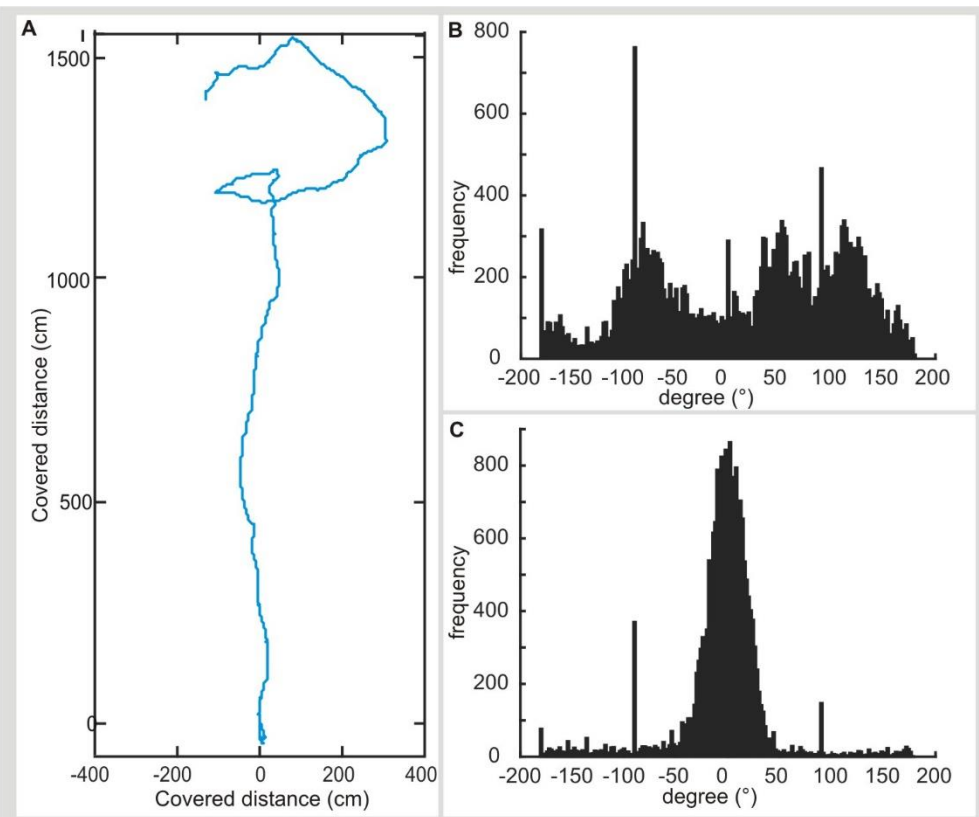
- **Interokulartransfer (IOT)** von **OF Information**
- Kein **Informationstransfer** zw. **Pedometer** und **OF-Meter**

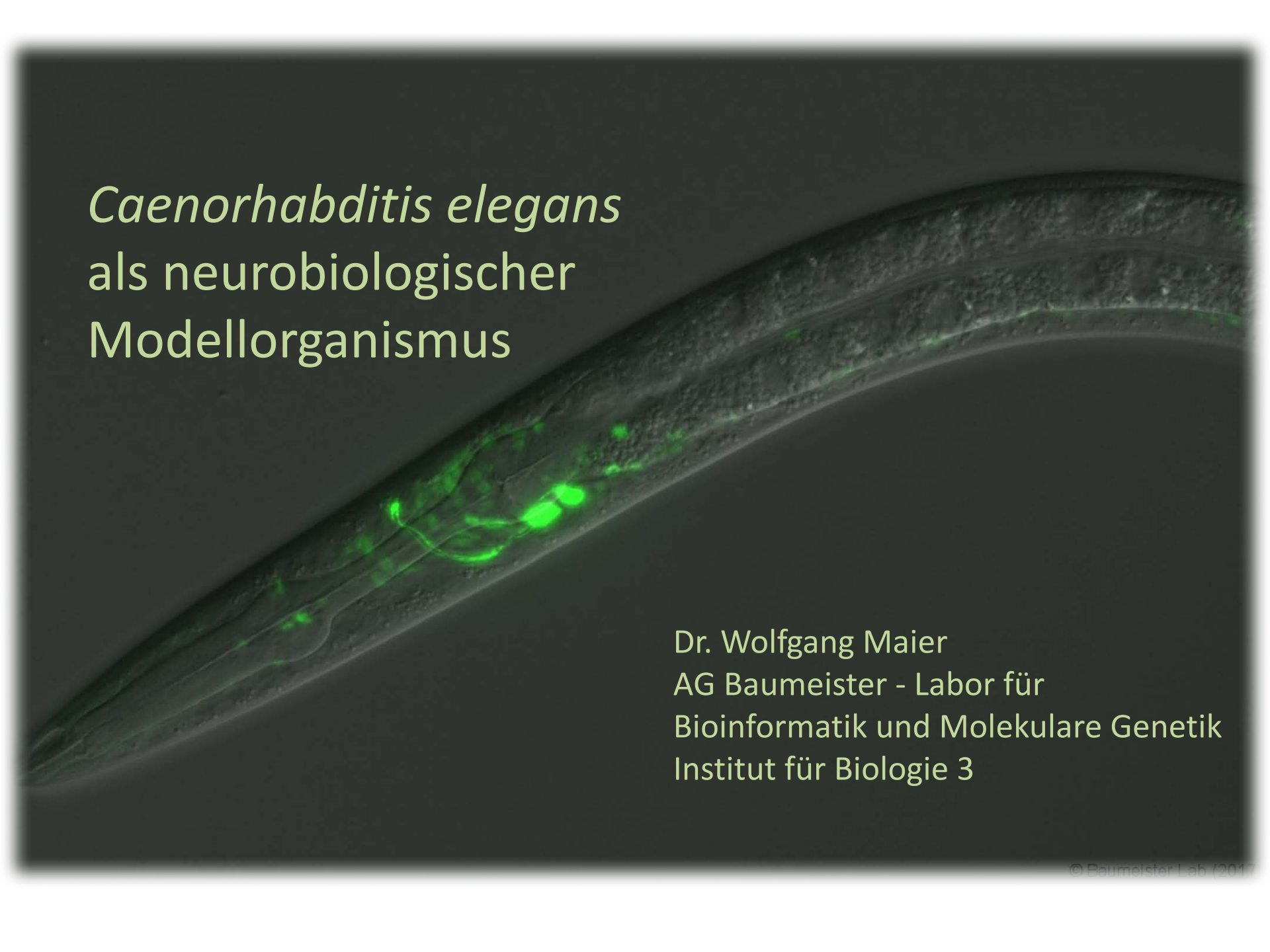






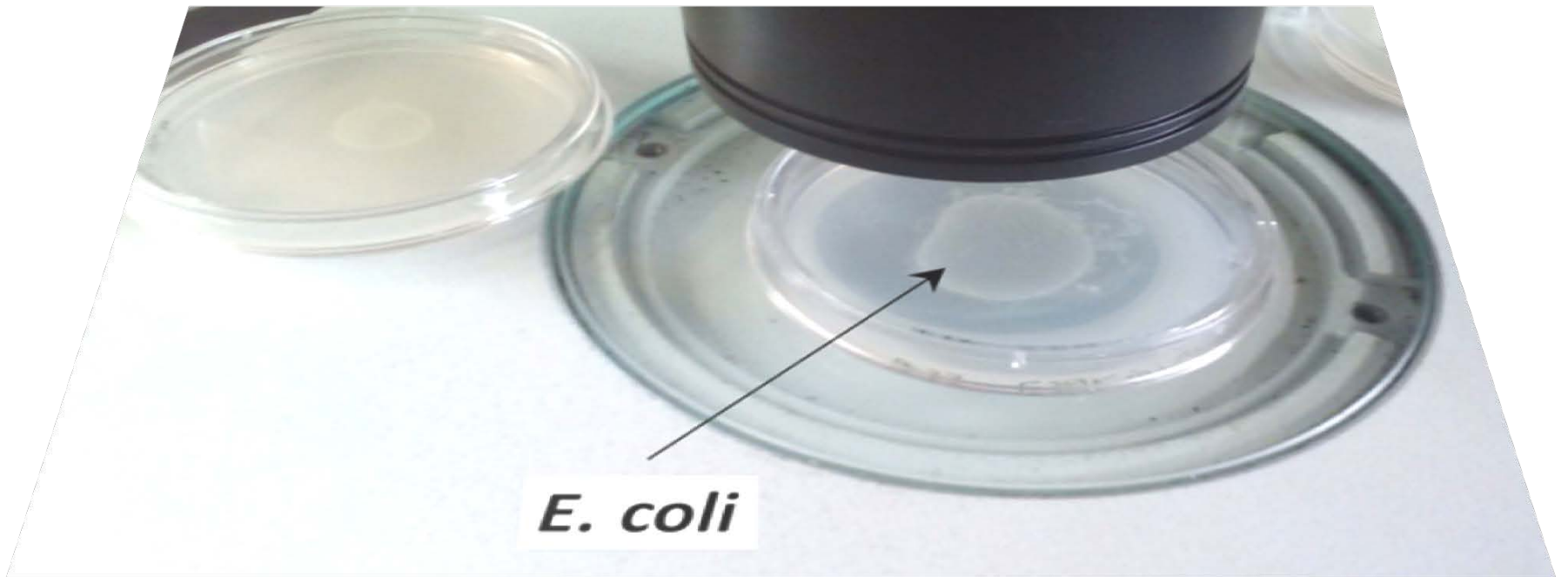
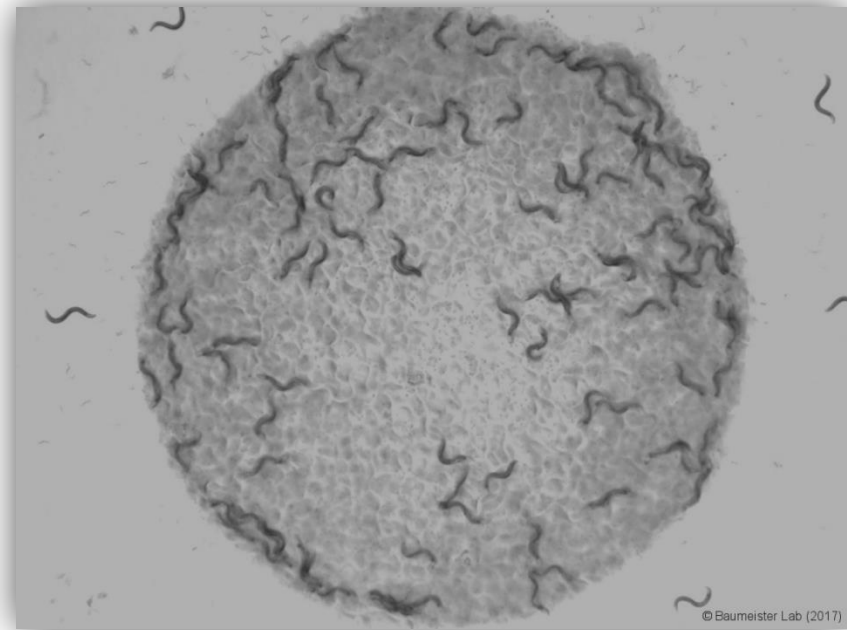
Laufkugelapparatur - Wegintegration

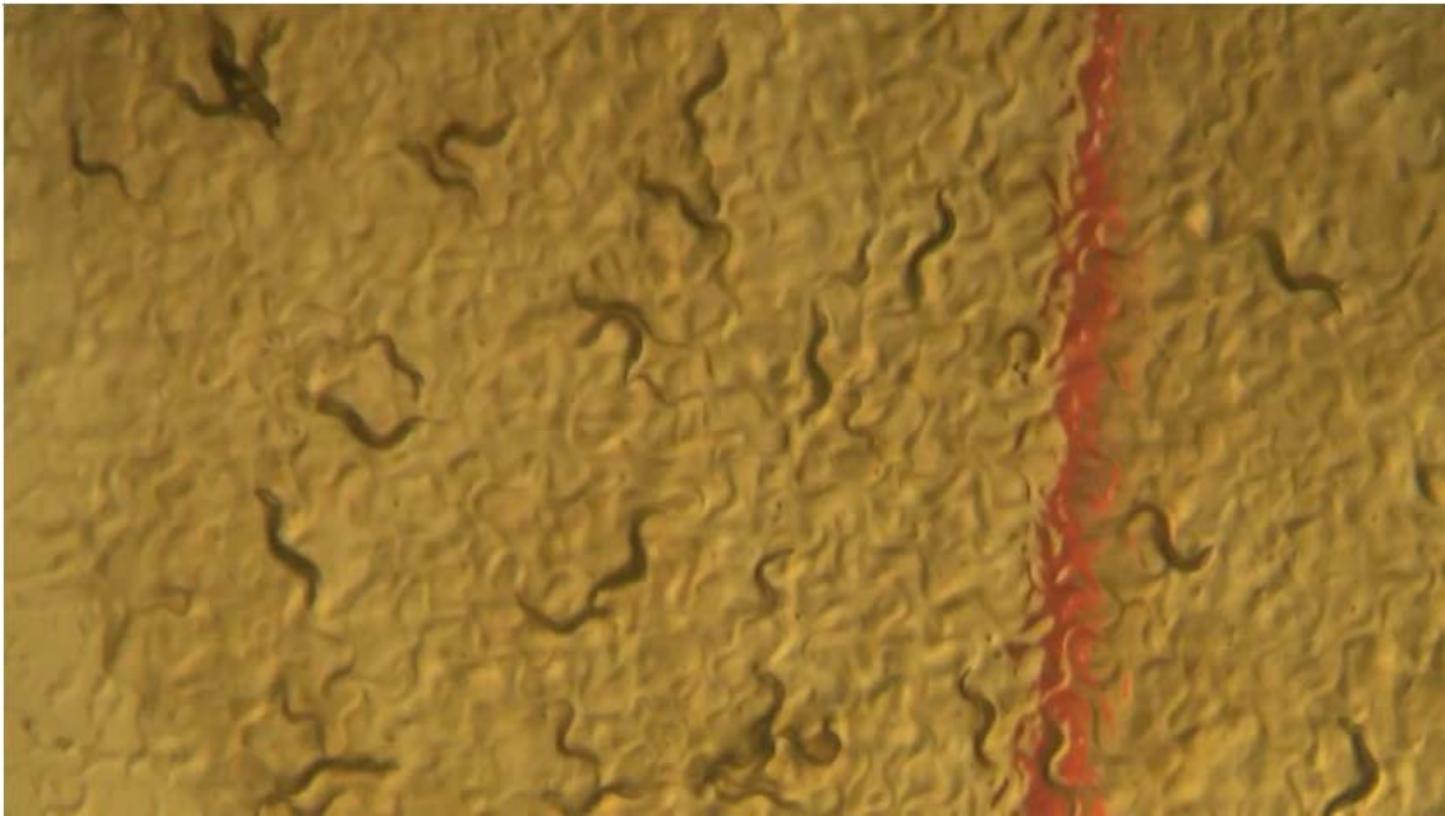




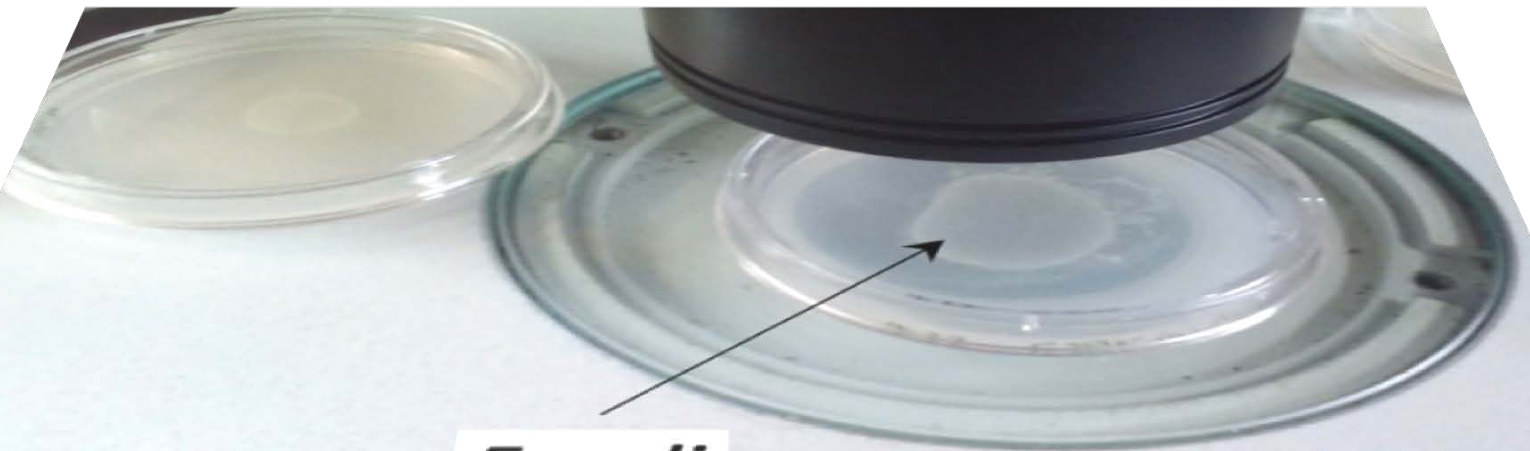
Caenorhabditis elegans
als neurobiologischer
Modellorganismus

Dr. Wolfgang Maier
AG Baumeister - Labor für
Bioinformatik und Molekulare Genetik
Institut für Biologie 3

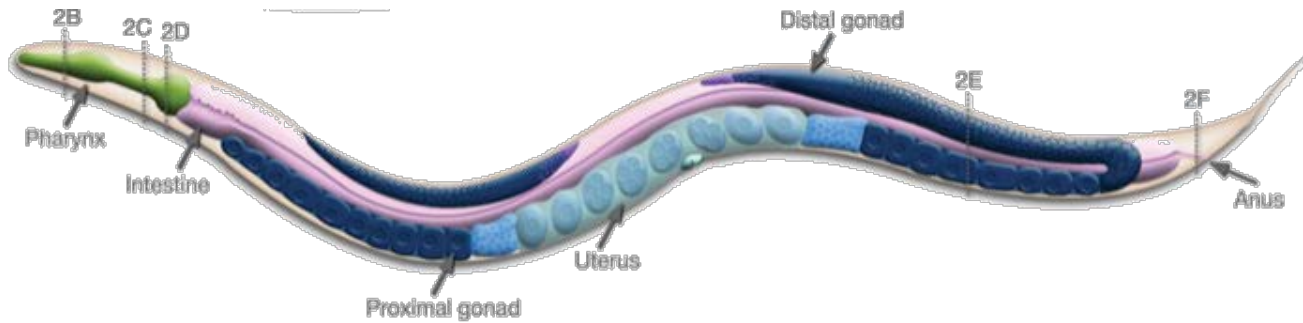
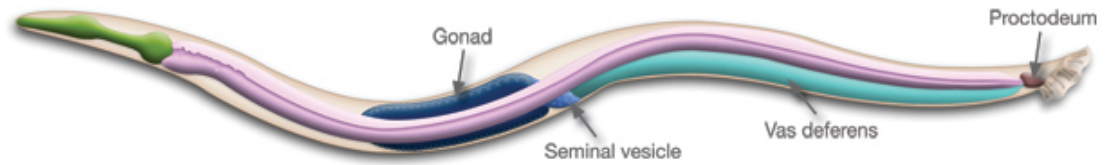
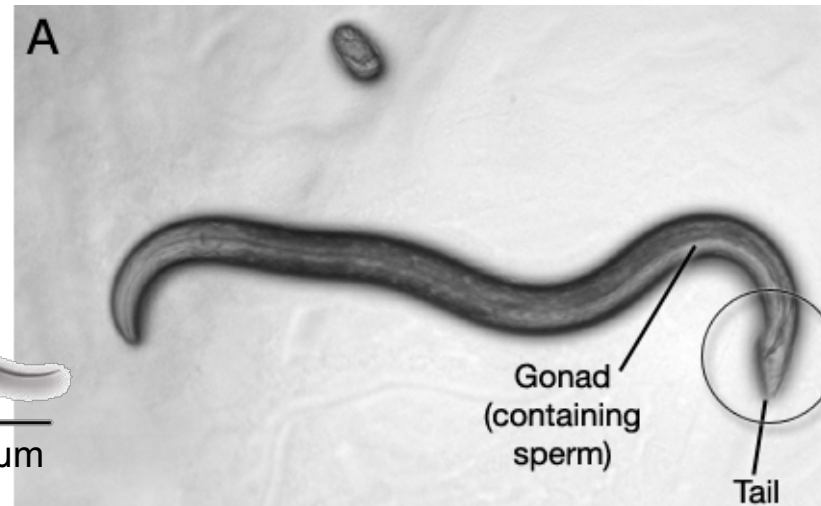




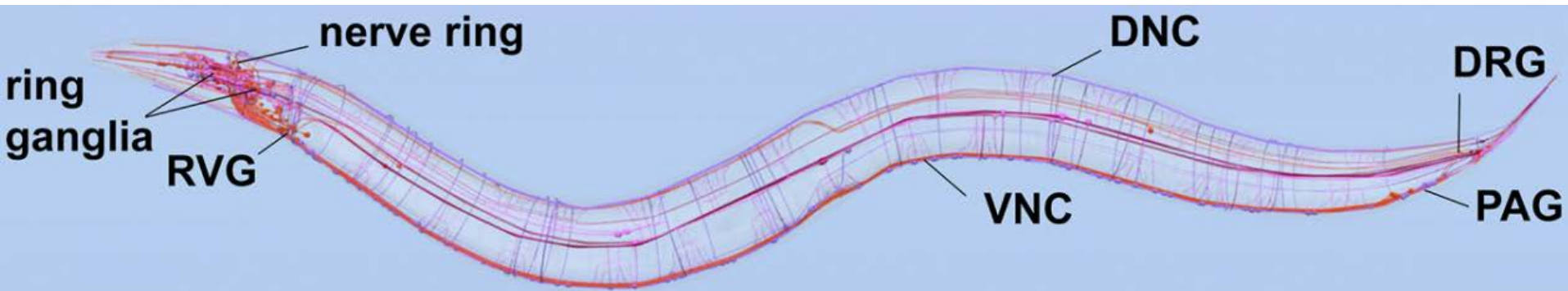
C. elegans Handhabung – Video unter <https://www.youtube.com/watch?v=llklqPULENk&feature=youtu.be>



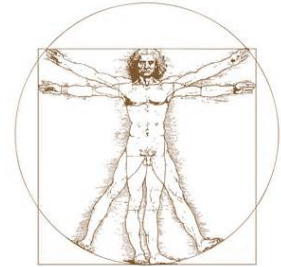
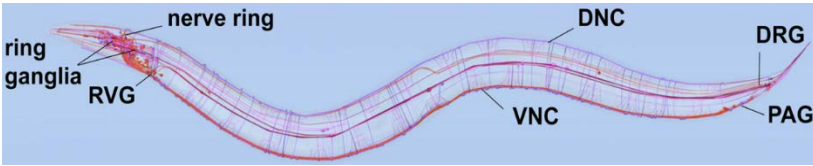
E. coli



Neuroanatomie



C. elegans - Neuroanatomie



302 Neuronen, 56 Gliazellen

6400 chemische Synapsen

900 Gap junctions

1500 neuromuskuläre Synapsen

Konnektom ~75% identisch

zwischen je 2 Individuen

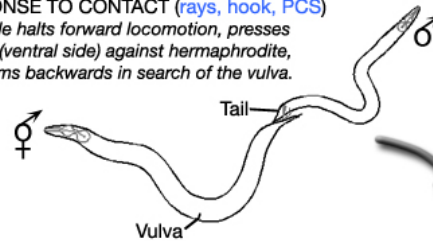
~ 100 000 Neuronen

~ 86 000 000 000 Neuronen

?? Synapsen ~ 100 000 000 000 000 Synapsen

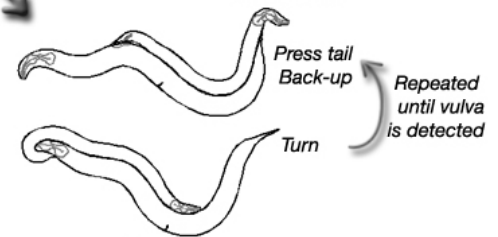
RESPONSE TO CONTACT (rays, hook, PCS)

Male halts forward locomotion, presses tail (ventral side) against hermaphrodite, swims backwards in search of the vulva.



TURNING (rays)

If the male reaches the end without detecting the vulva, a tight turn is executed and searching continues along the other side.



VULVAL LOCATION

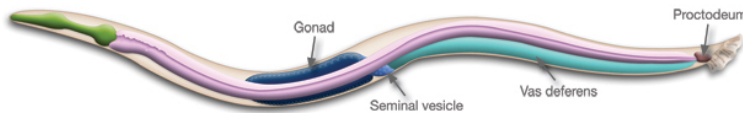
If the male detects the vulva (hook, PCS), he commences a slow search of the area, prodding for the slit with his spicules.



SPICULE INSERTION
SPERM TRANSFER

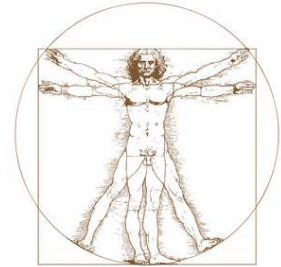
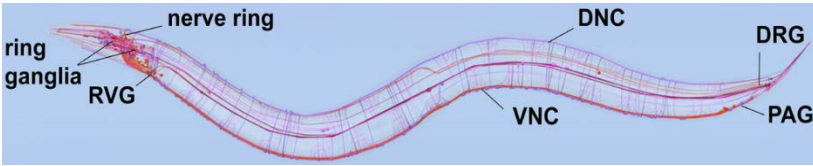
The male inserts spicules fully into the vulva, locking himself to the hermaphrodite and ejaculates

Tail sensory structures (blue) required for this step.



383 Neuronen, 92 Gliazellen

C. elegans - Neuroanatomie



302 Neuronen, 56 Gliazellen

6400 chemische Synapsen

900 Gap junctions

1500 neuromuskuläre Synapsen

Konnektom ~75% identisch
zwischen je 2 Individuen

~ 100 000 Neuronen

~ 86 000 000 000 Neuronen

?? Synapsen ~ 100 000 000 000 000 Synapsen



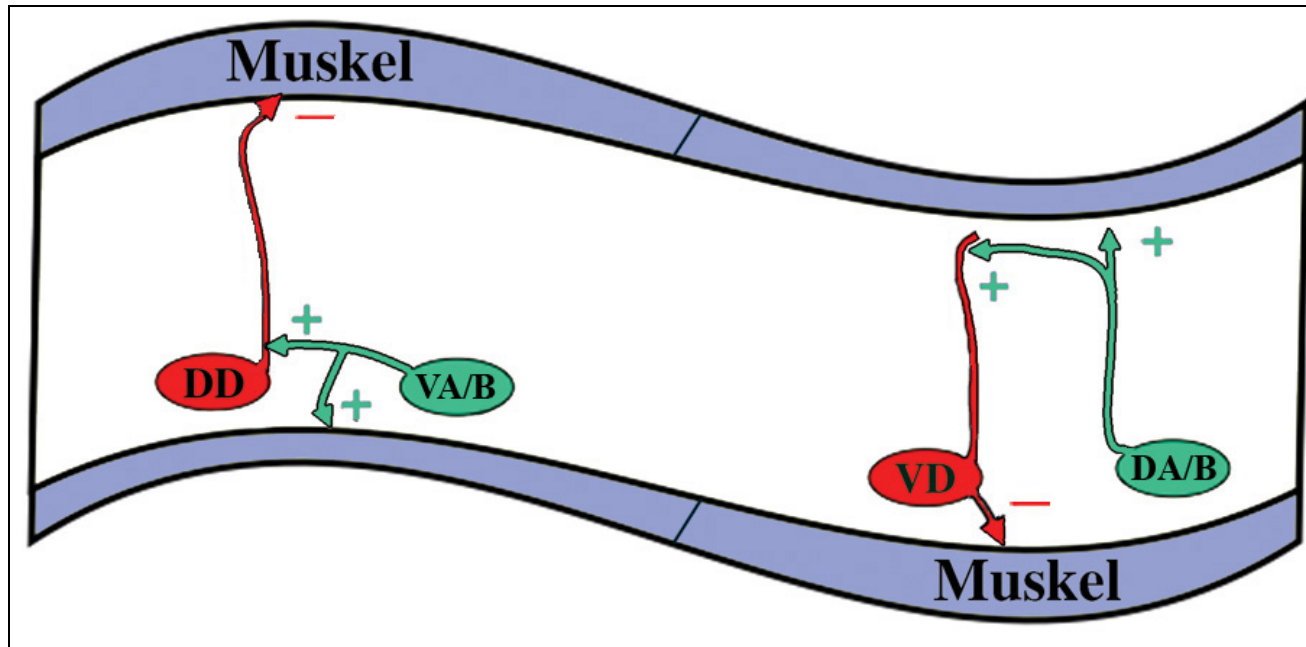
Vision der 1970er Jahre: Computermodell des Nervensystems

→ „The Mind of a Worm“ (White et al., 1986)

Karte des gesamten Nervensystems inklusive aller Synapsen

→ aber: große Schwierigkeiten bei der **funktionellen** Vermessung

Grunds Schaltkreis der Lokomotion

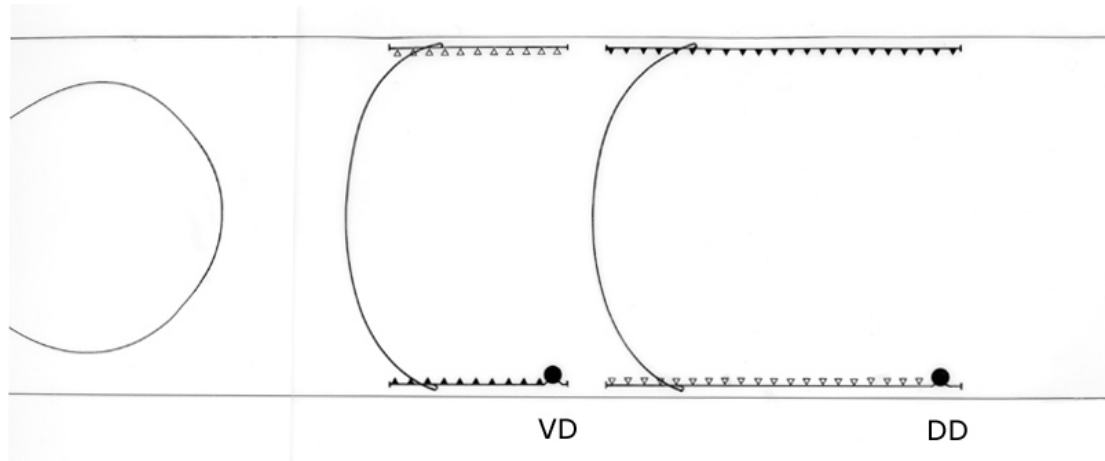
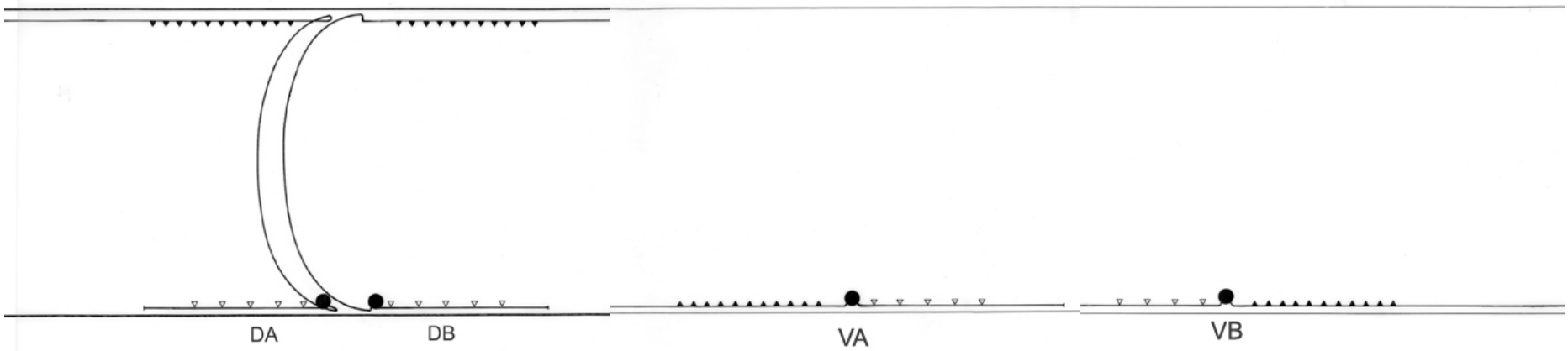
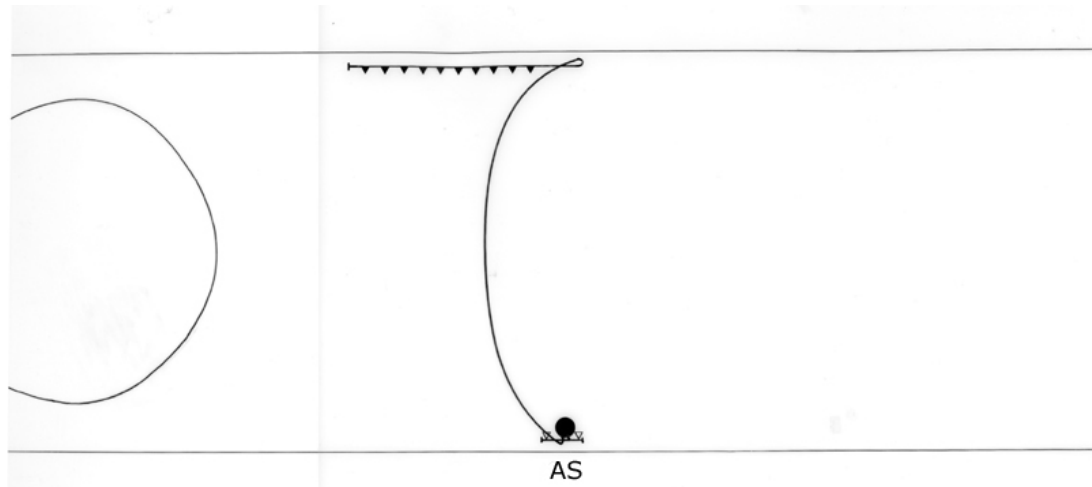


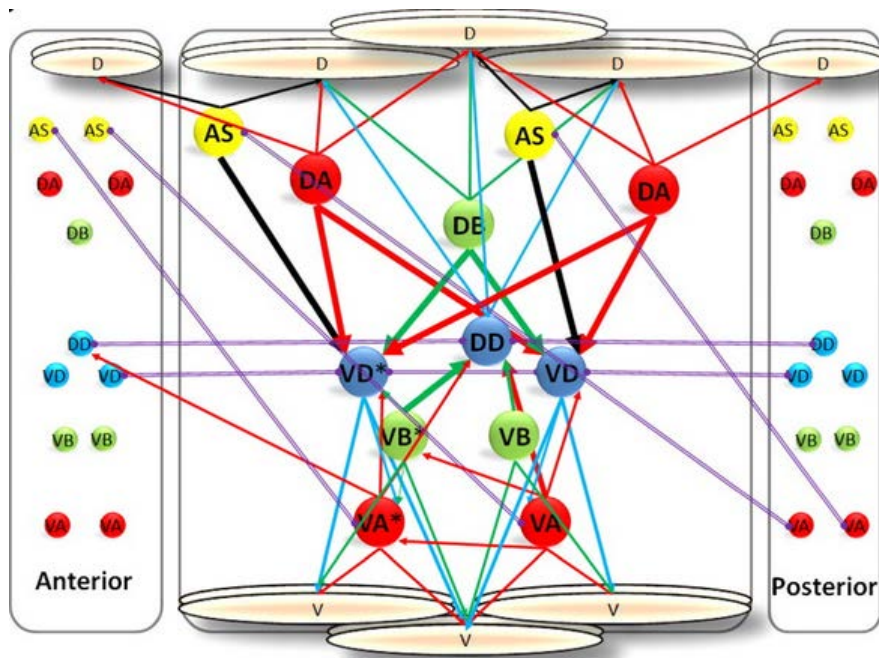
● = GABAergic

● = cholinergic

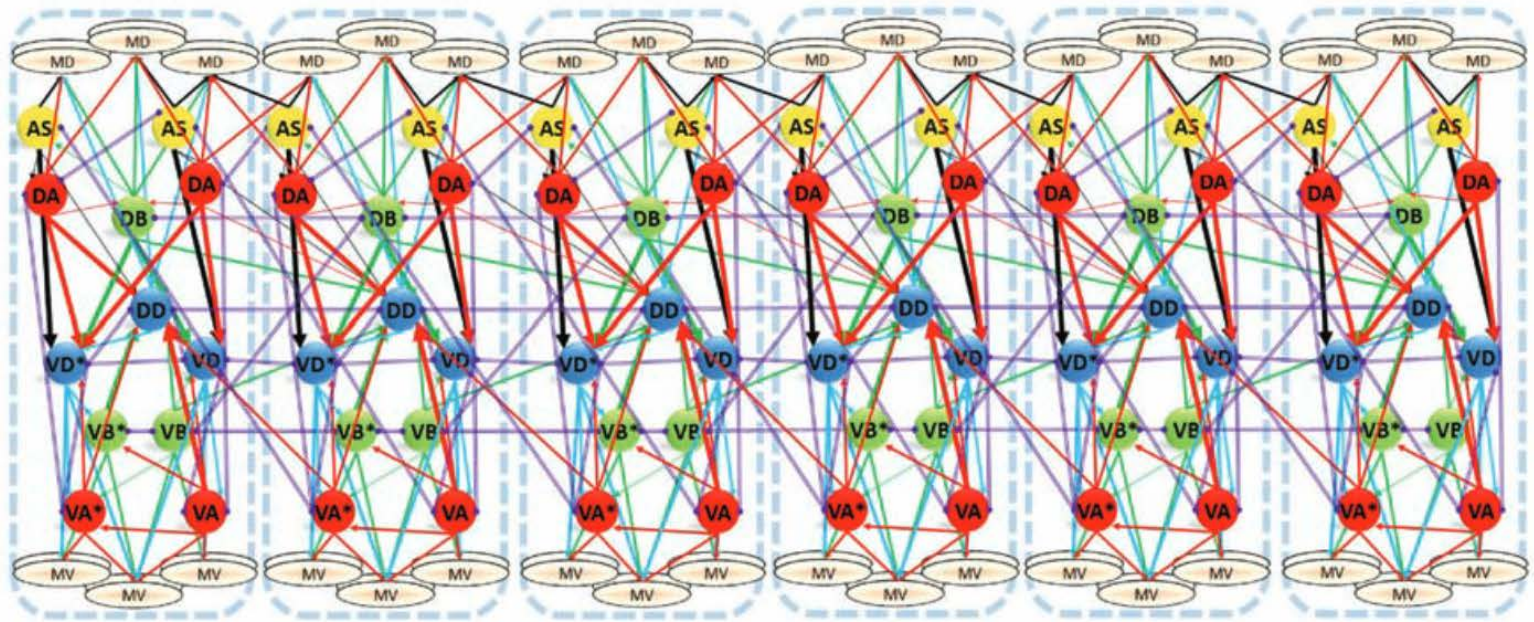
Reziproke Inhibition

Grundschaltkreis der Lokomotion

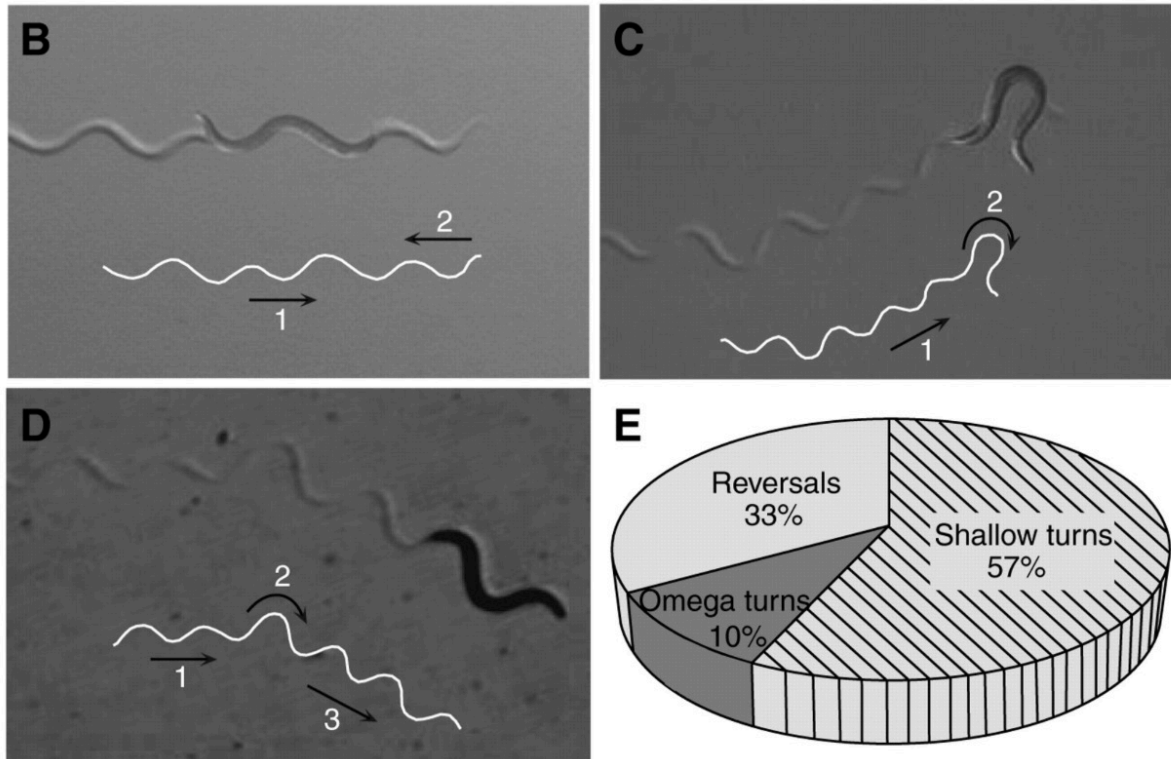




Grunds Schaltkreis der Lokomotion

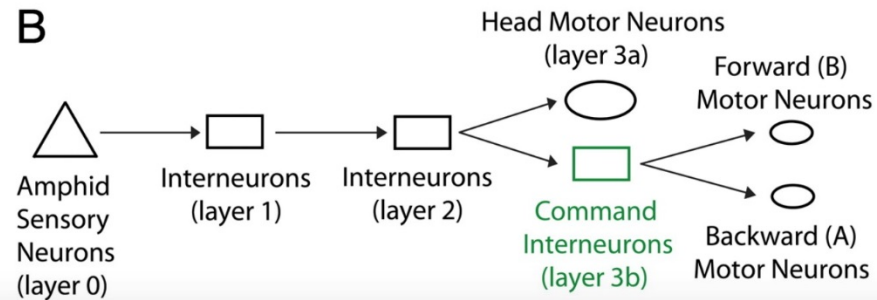
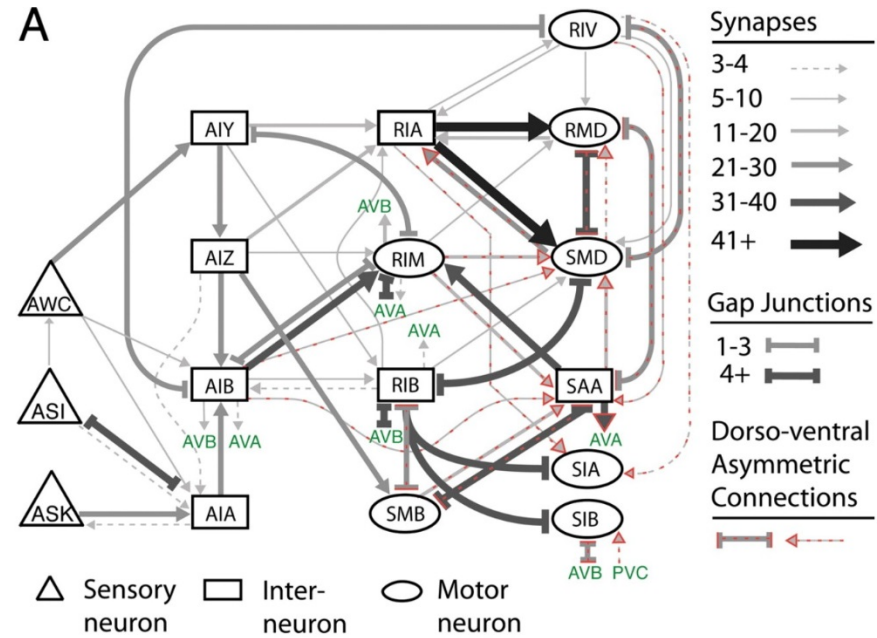
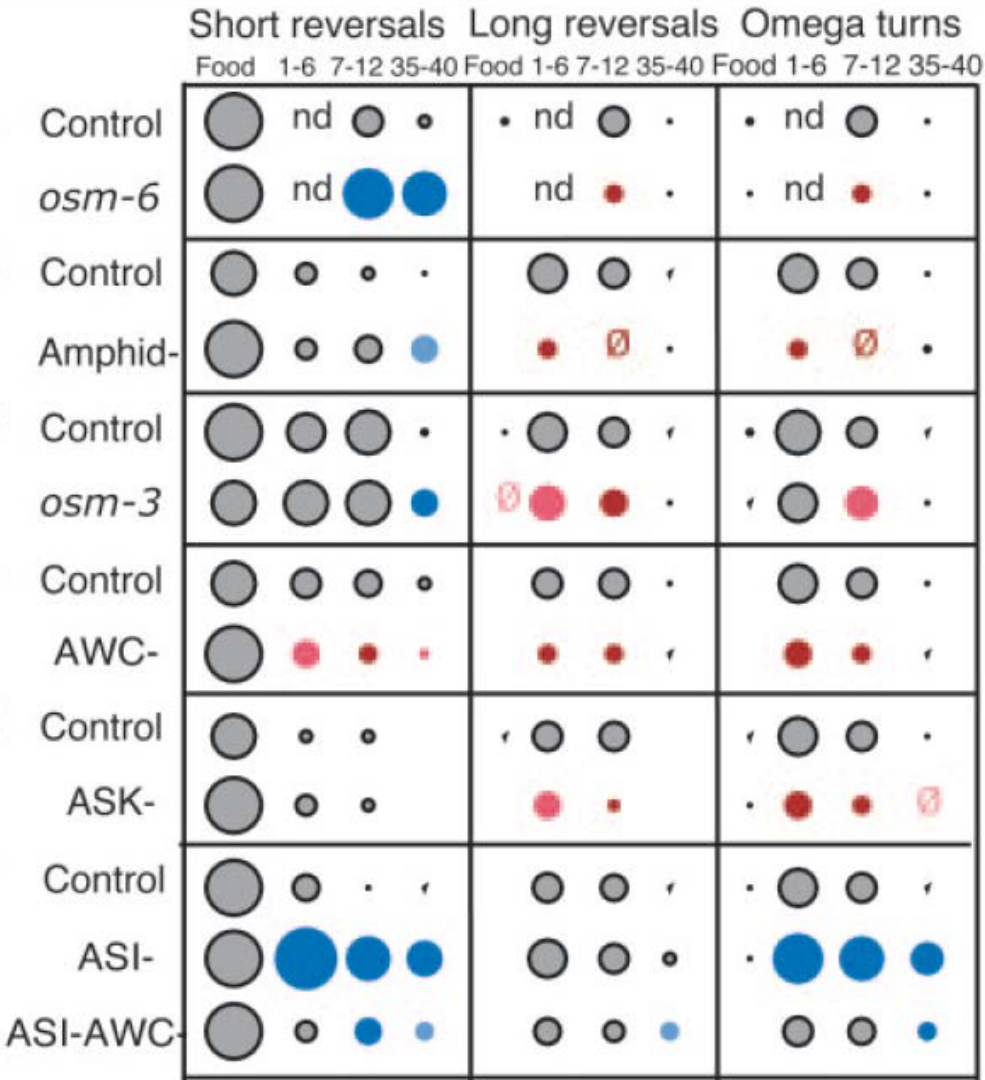


Schaltkreis „echter“ Lokomotion



(Kim et al., 2011)

Schaltkreis „echter“ Lokomotion



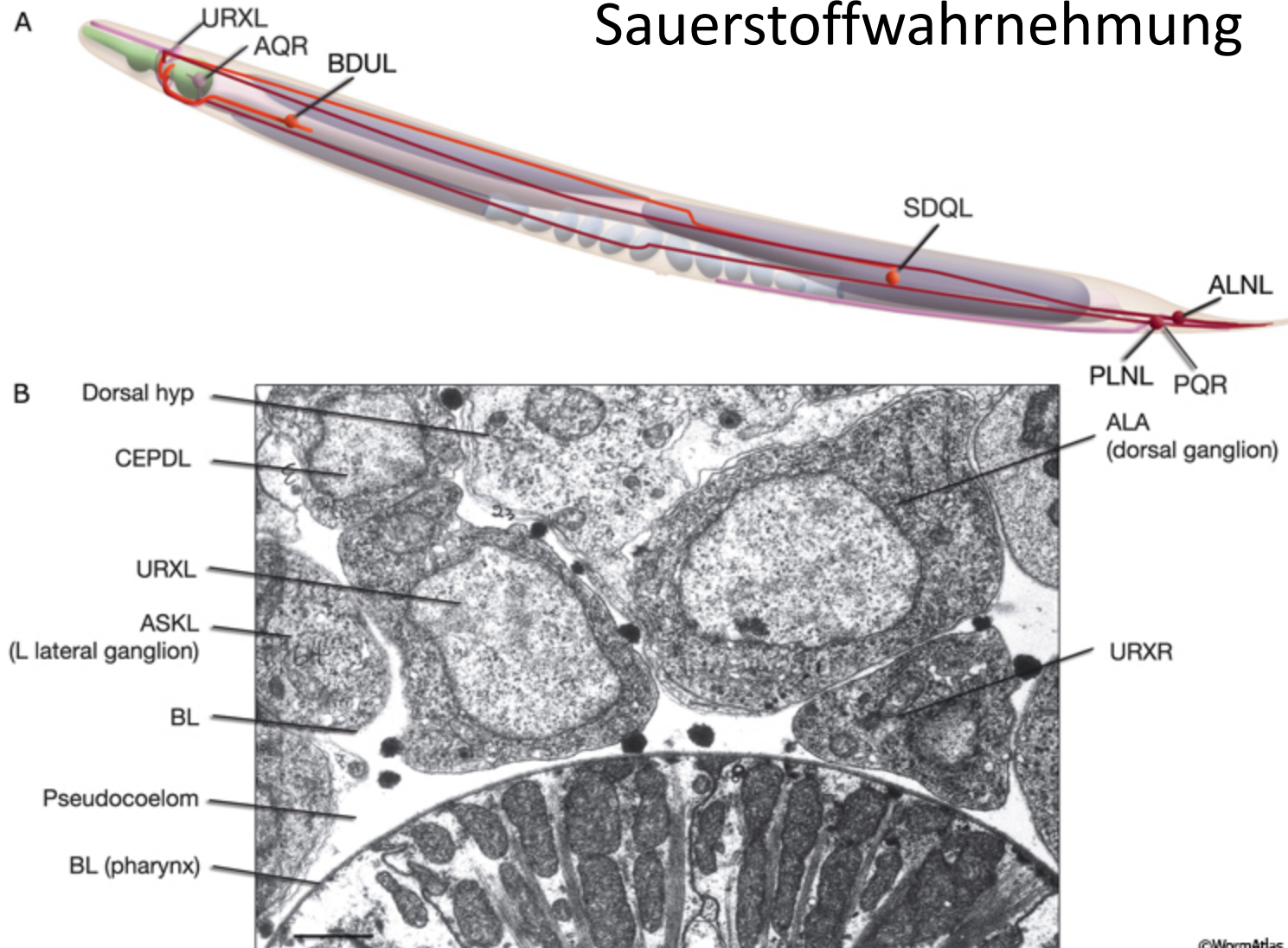
(Gray et al. 2005)

Sensorischer Input verändert Verhalten

Welche Umweltreize
verarbeitet ein Wurm überhaupt?

Sensorischer Input verändert Verhalten

Sauerstoffwahrnehmung



Sensorischer Input verändert Verhalten

Oberflächenbeschaffenheit
(auch Biofilme)

Temperaturgradienten

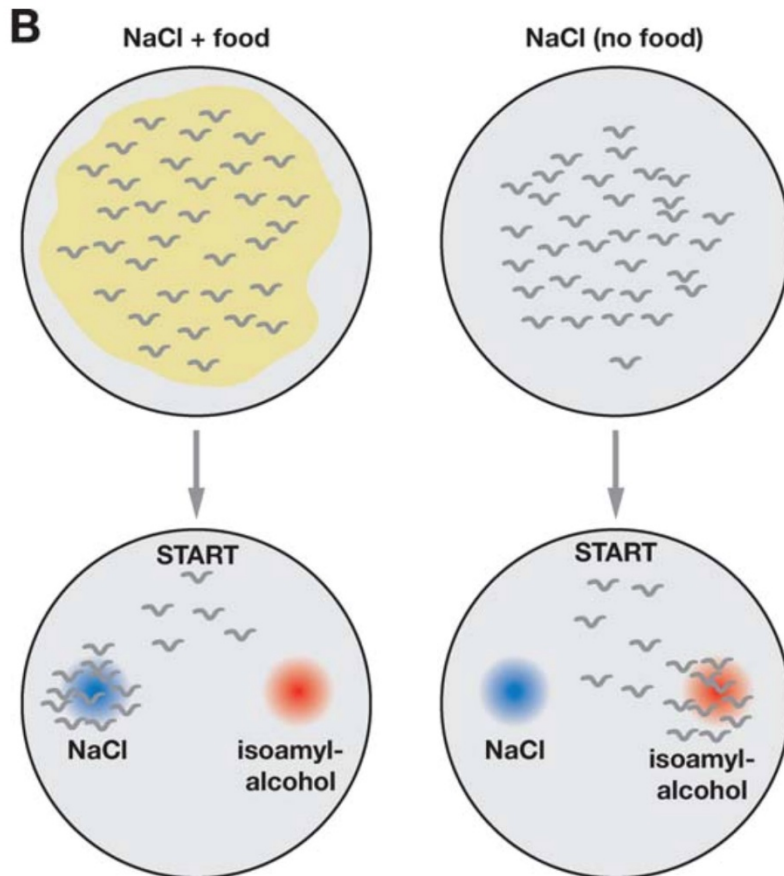
O_2
 CO_2
 H_2S

elektromagnetische Felder

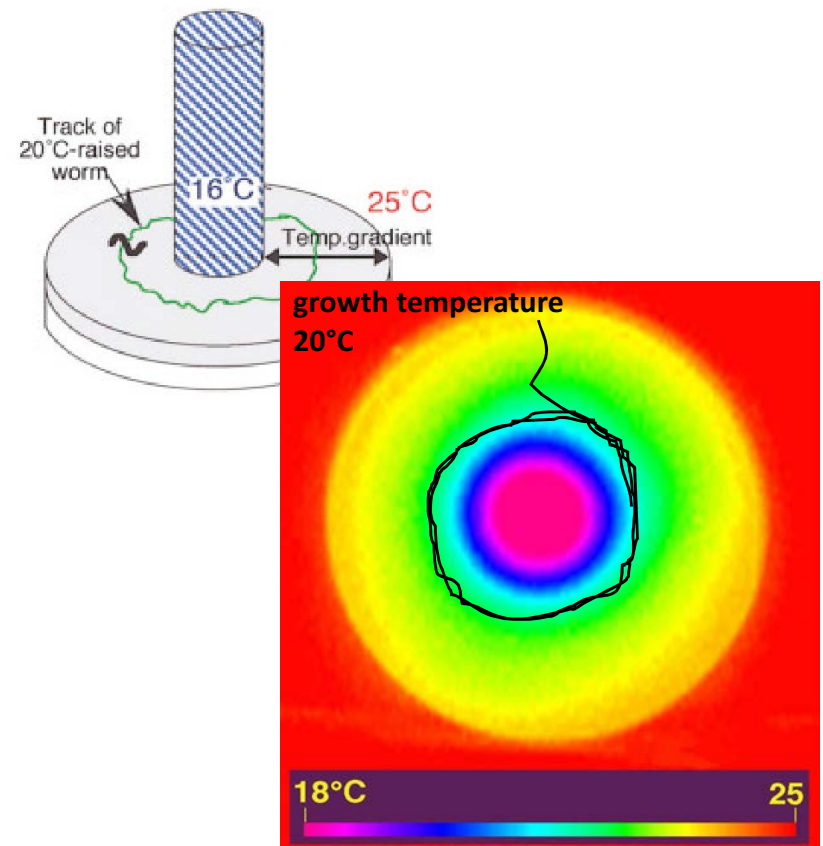
Pheromone

Lernen ändert Verhalten auch im Wurm

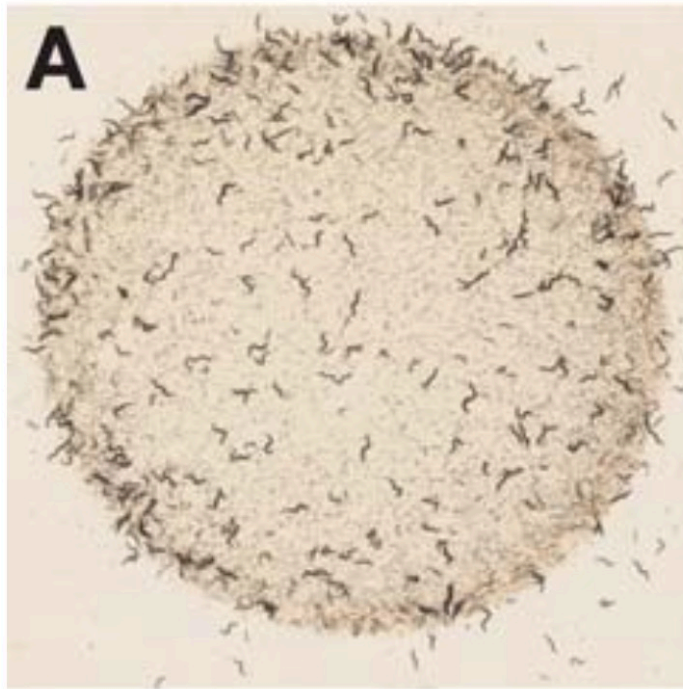
Chemotaxis



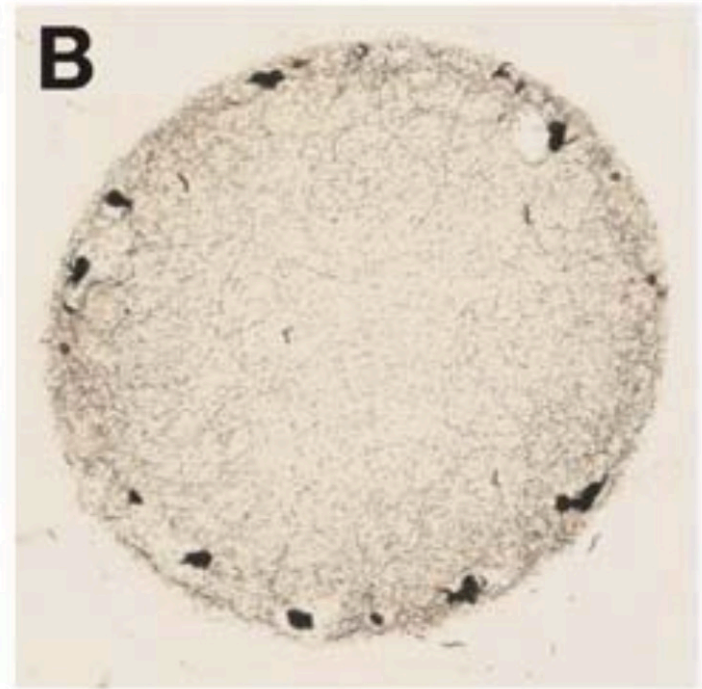
Thermotaxis



Neuropeptide – Modulation von Verhaltensweisen



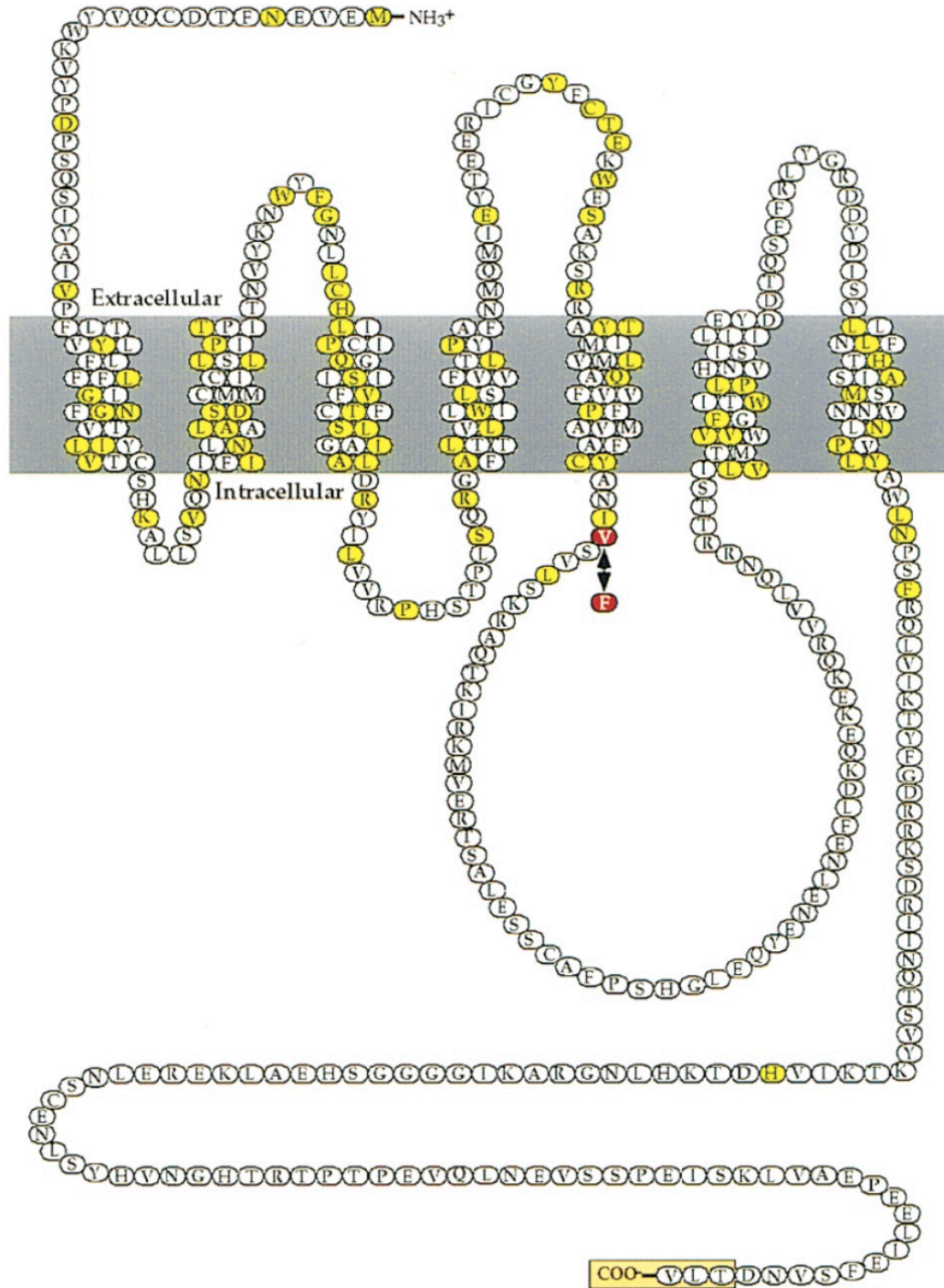
solitary



social

feeding

(de Bono & Bargmann, 1998)



Neuropeptidrezeptor-1 (NPR-1)

molekulare Determinante des
Freßverhaltens

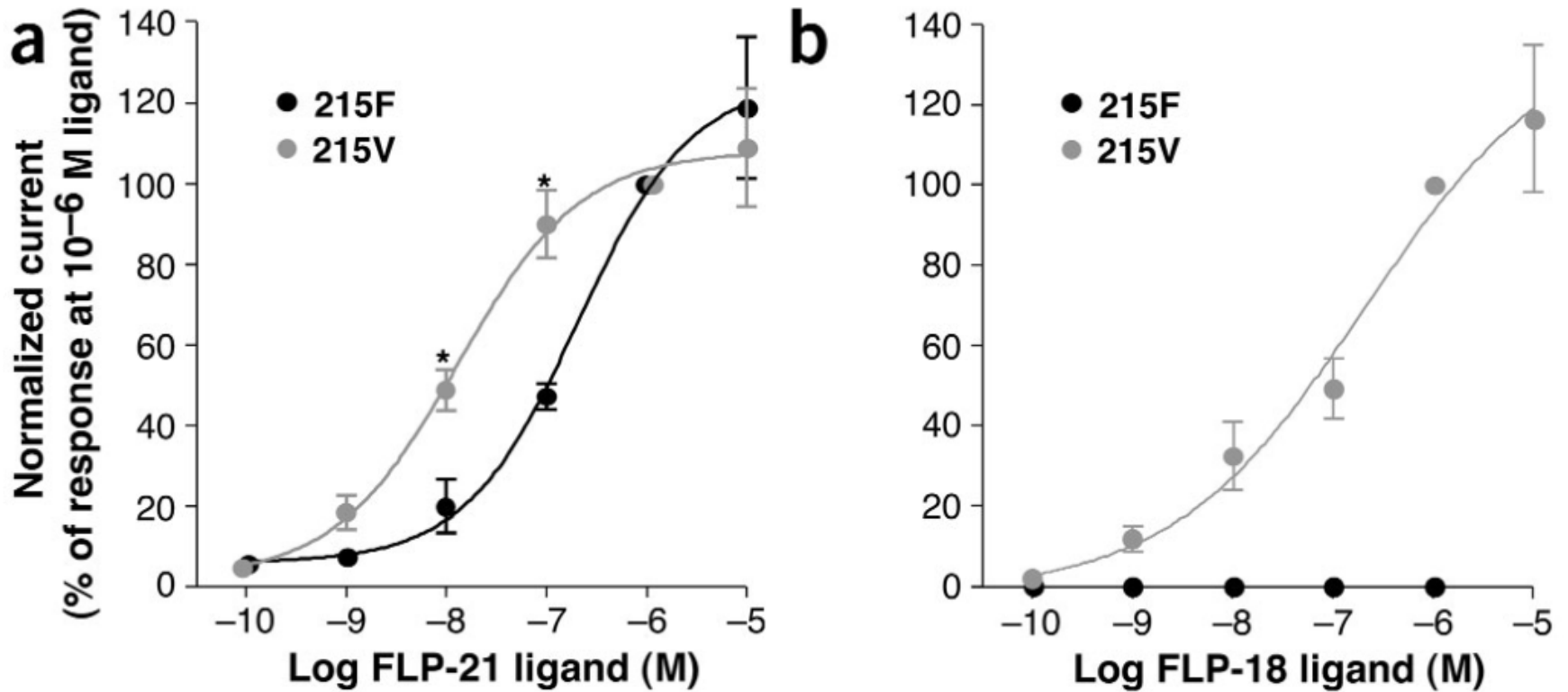
Würmer mit **Valin** an **Position 215** des
7-Transmembrandomänen-Proteins
sind **“solitary feeder”**, solche mit
Phenylalanin sind **“social feeder”**

“normale” Laborwürmer tragen die
Valin-Variante und sind deshalb
Einzelgänger

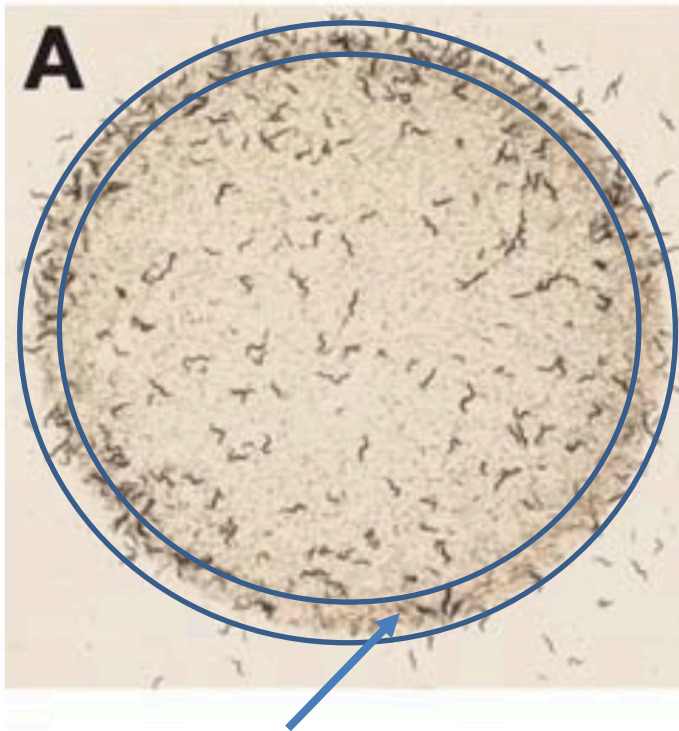
NPR-1 ist der molekulare Rezeptor für
die Neuropeptide FLP-18 und FLP-21

(de Bono & Bargmann, 1998)

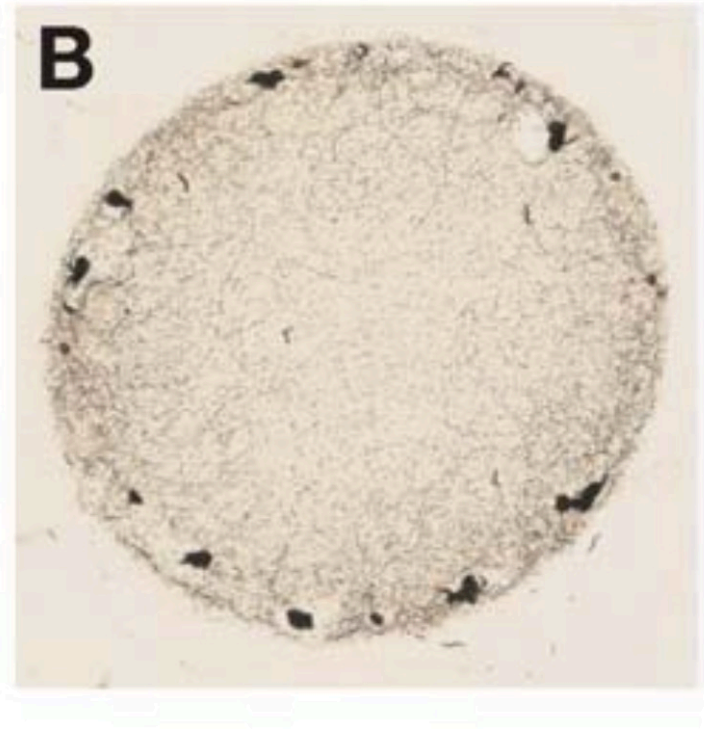
Die Valin-Variante von NPR-1 antwortet stärker auf FLP-21 und FLP-18



Gemeinschaftssinn in Würmern?

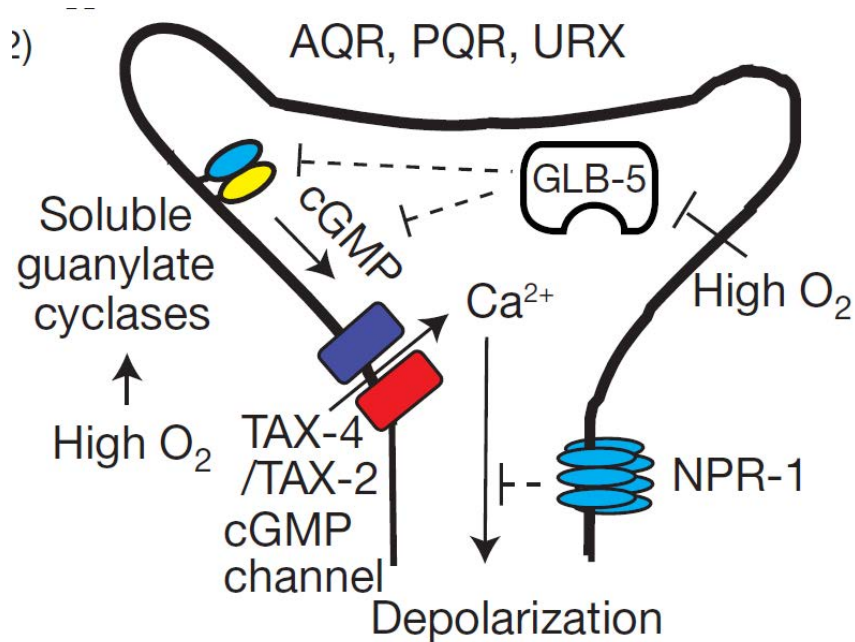


etwas dickere Bakteriensicht am Rasenrand -> mehr Stoffwechsel -> weniger O₂

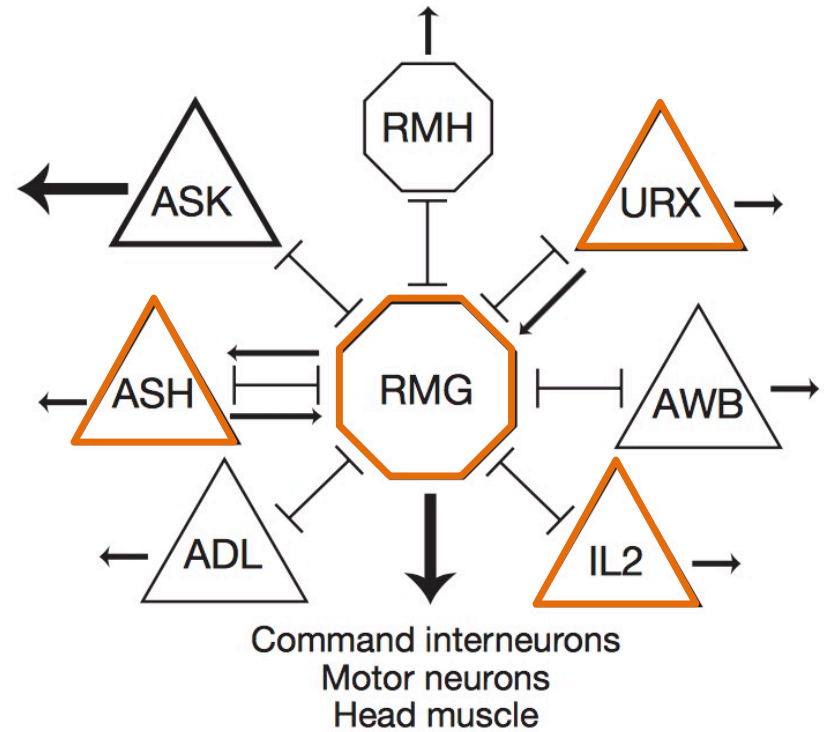


Würmer mit O₂-Aversion sammeln sich in den Randbereichen -> Selbstverstärkung des Effekts durch Wurmmetabolismus

NPR-1 moduliert einen Schaltkreis zur O₂-Vermeidung



(Persson et al., 2009)



—|—| Gap junction

→ Chemical synapse

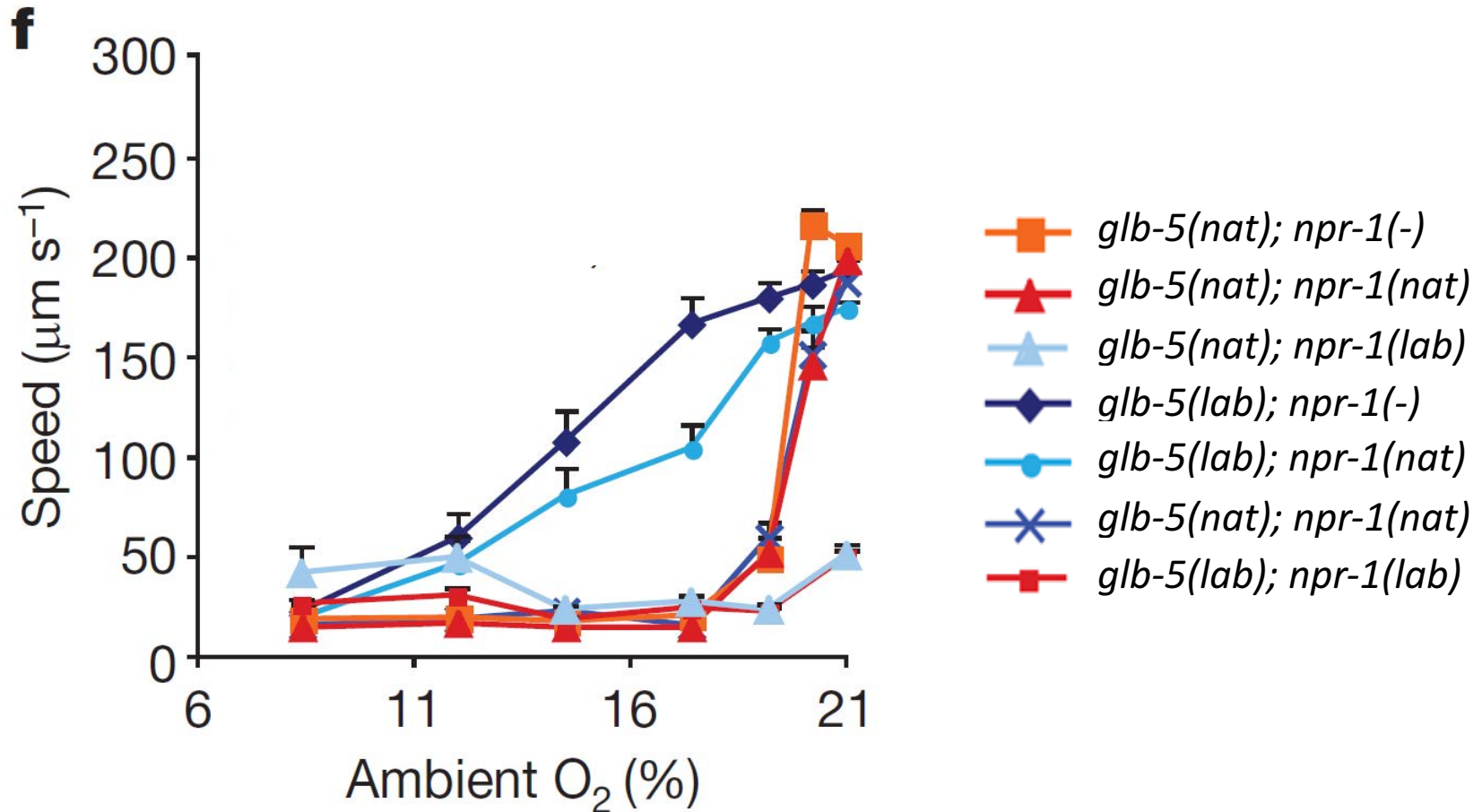
△ Sensory neuron

○ Inter/motor neuron

Expression von NPR-1

(Macosko et al., 2009; Choi et al., 2013)

Anpassung eines komplexen Verhaltens durch nur zwei Punktmutationen

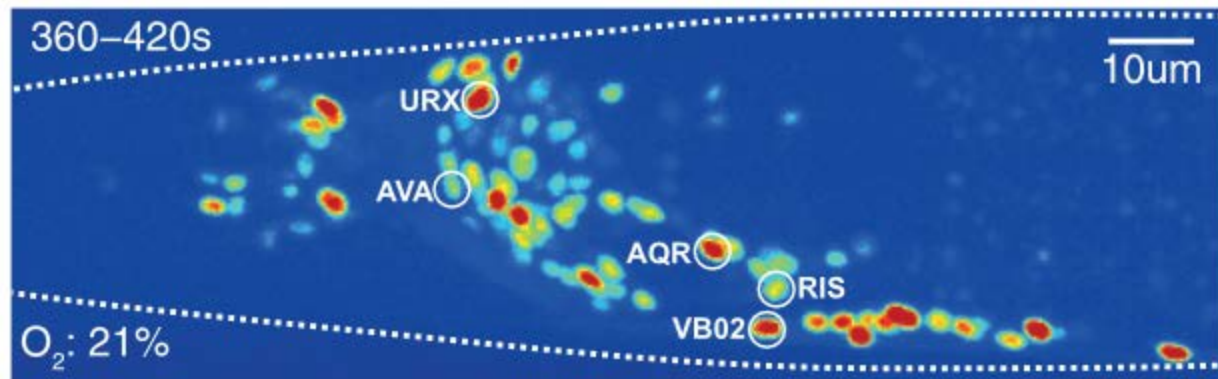
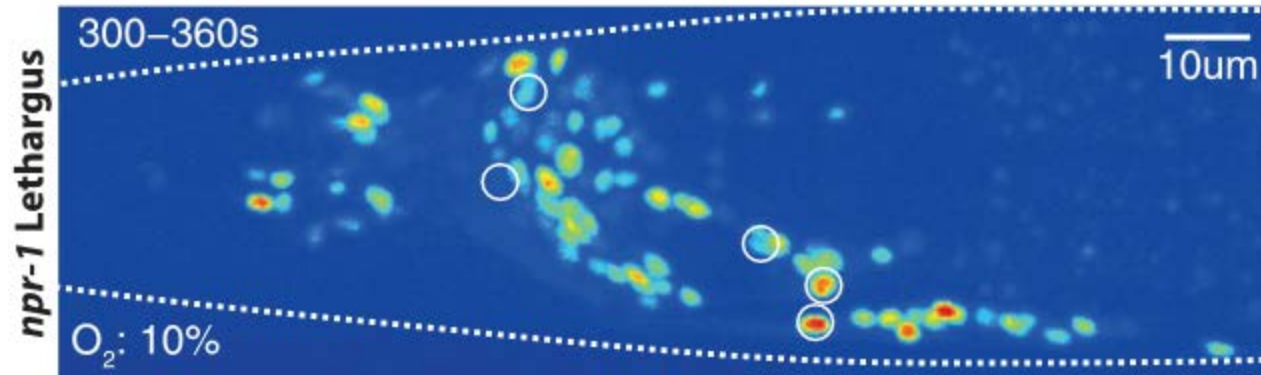


(Persson et al., 2009)

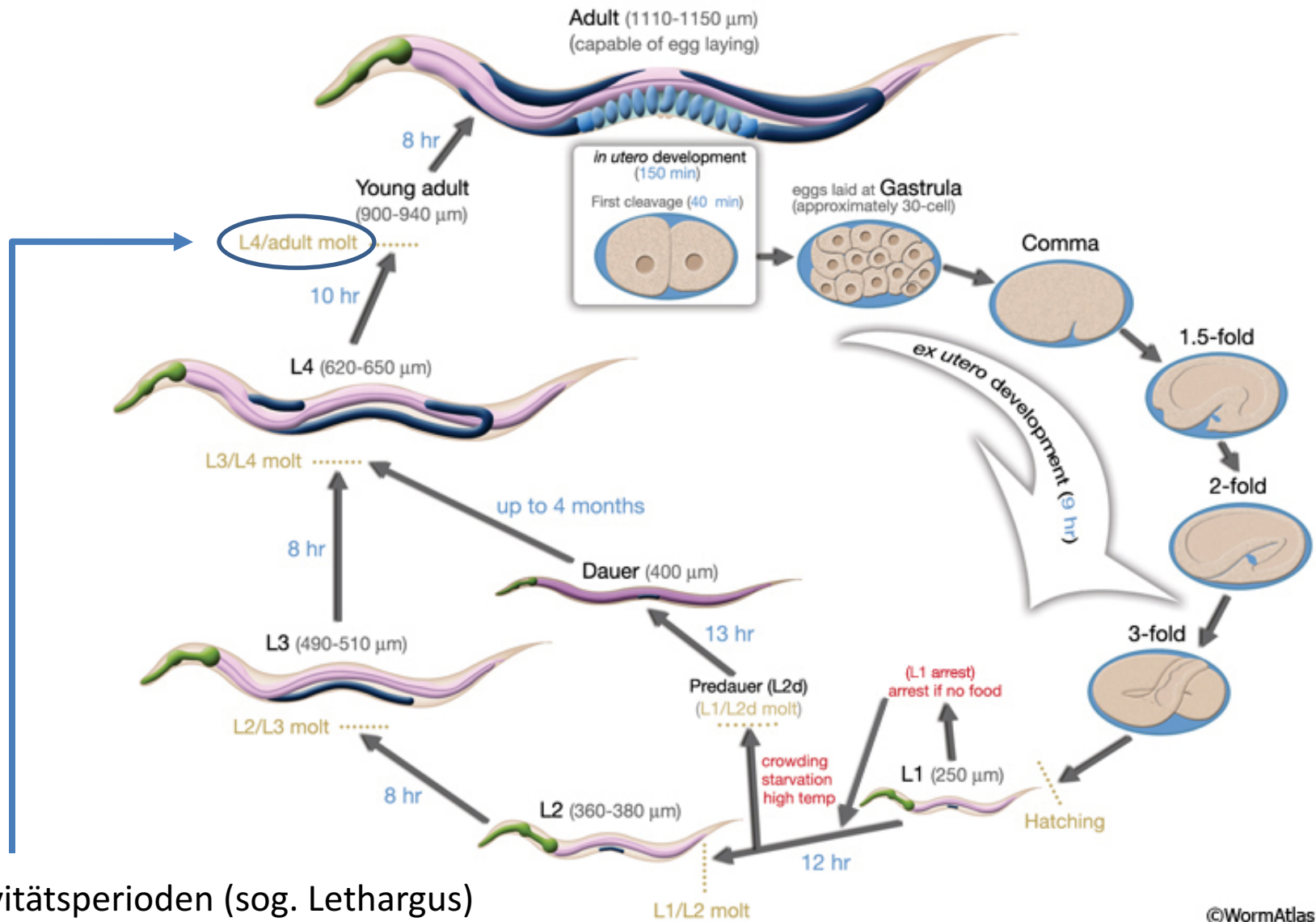
Moderne Bildgebungsverfahren

Live-Beobachtung von Aktivierungsmustern
im Wurm-Nervensystem

Grundlagen des “Schlafs” bei Würmern



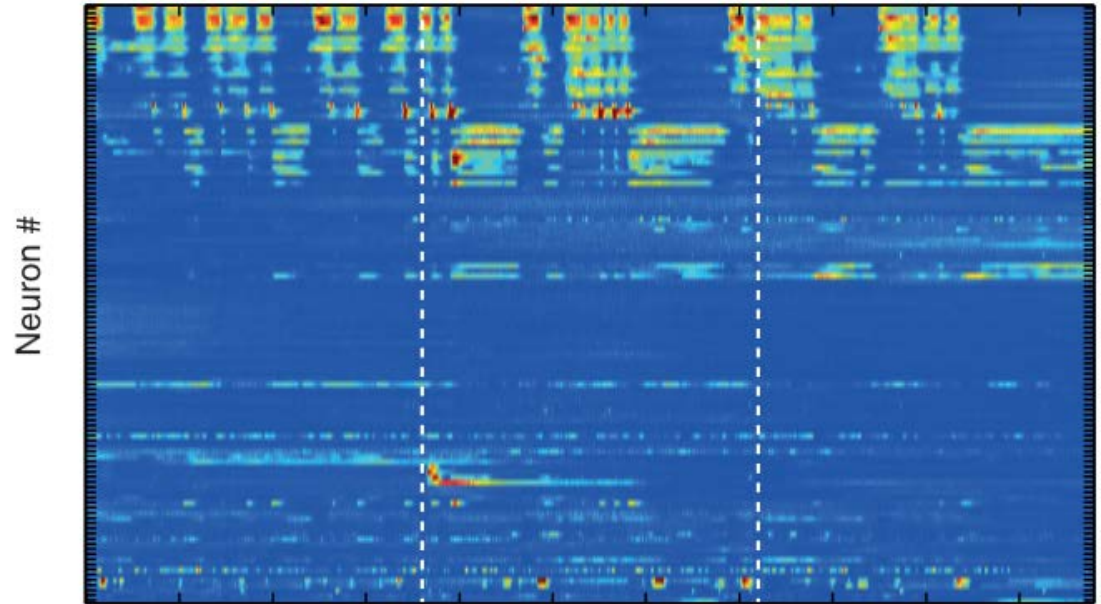
(Nichols et al., 2017)



Inaktivitätsperioden (sog. Lethargus) während der Häutung vom 4. Larvenstadium (L4) zum jungen adulten Tier dienen als „Schlaf“-Modell

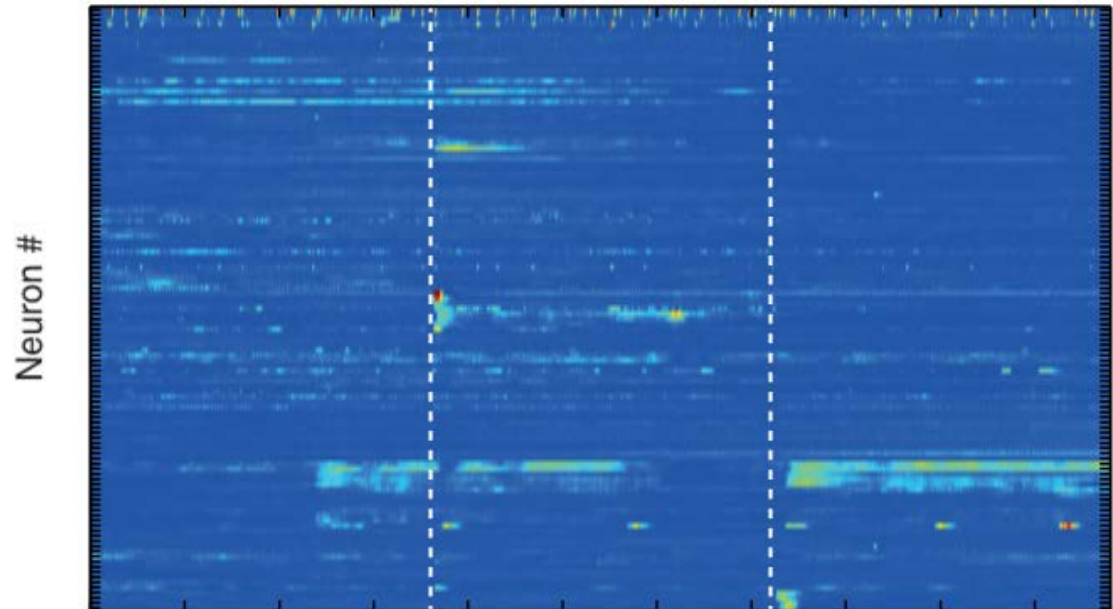
aktiver Wurm

normales L4-Aktivitätsmuster



ruhender Wurm

Aktivitätsmuster während des Lethargus-Zustands

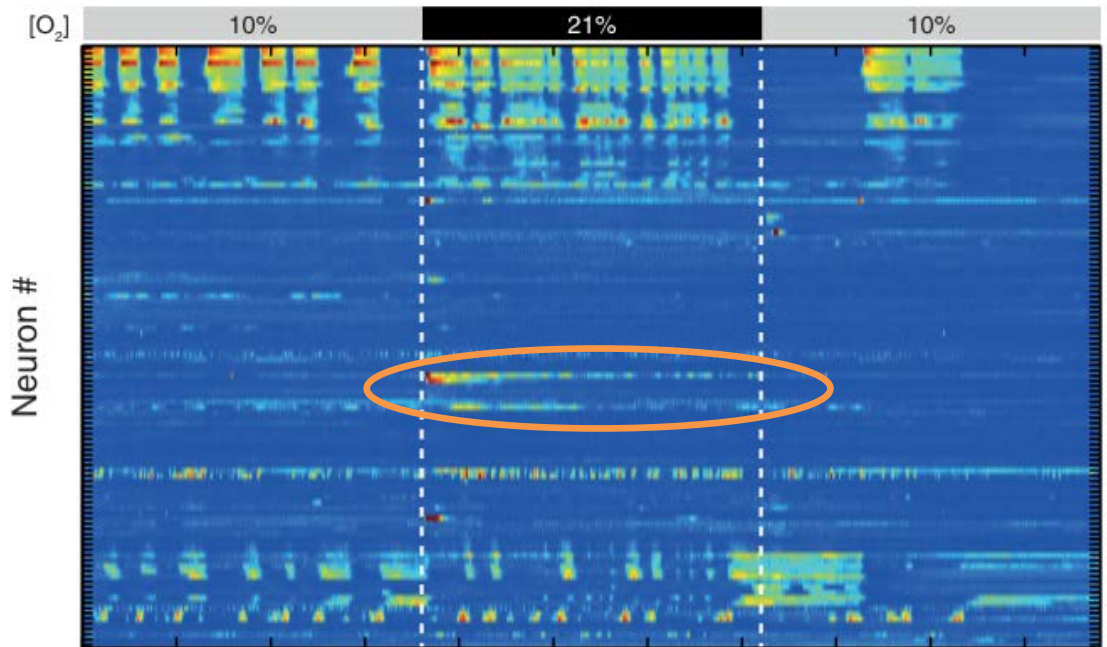


O₂-empfindliche social feeder Stämme
lassen sich durch Erhöhung der Sauerstoffkonzentration aufwecken

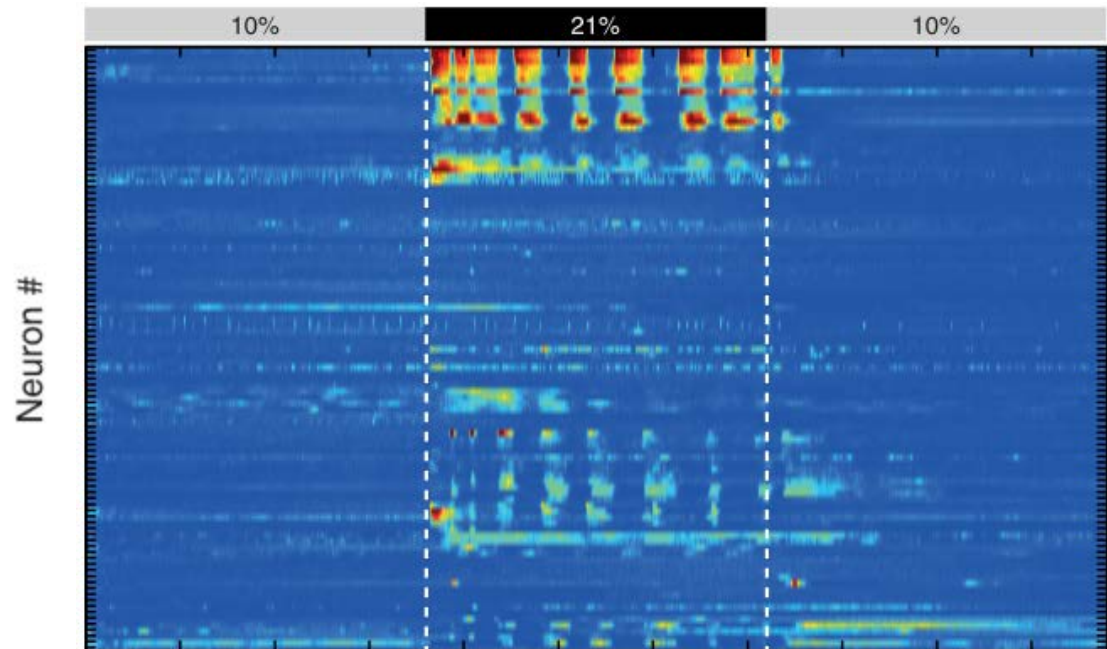
-

direkte Beobachtung eines erwachenden Nervensystems!

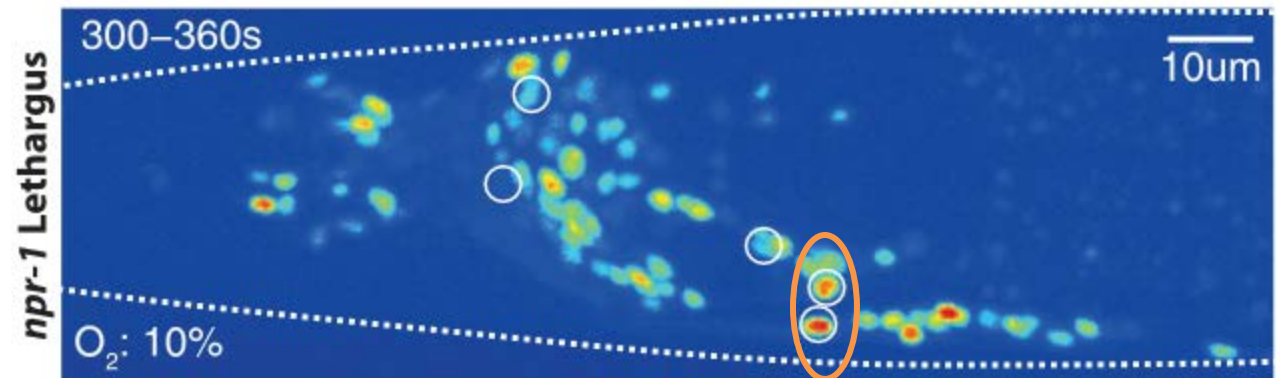
Im wachen L4-Tier verursacht eine Änderung der O_2 -Konzentration nur lokale Änderungen in der Nervenzellaktivierung (v.a. in primär sauerstoffwahrnehmenden Neuronen).



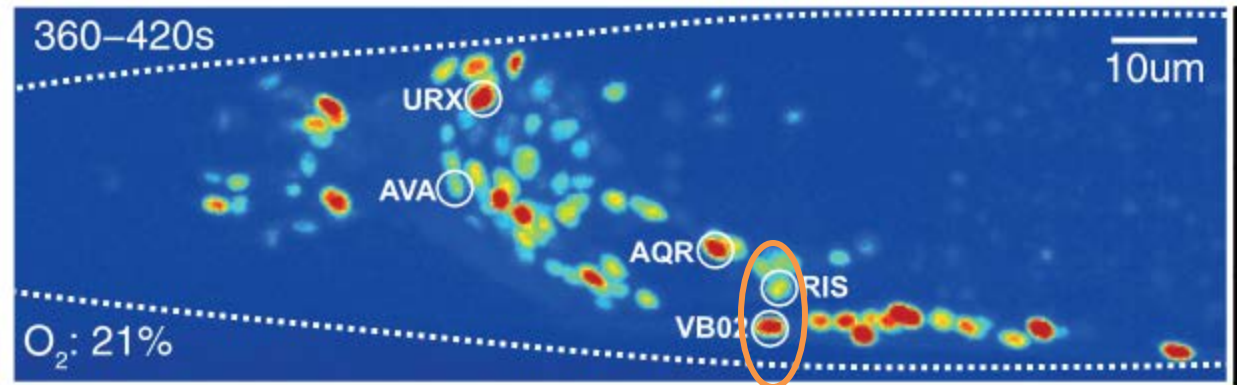
O_2 -vermitteltes Aufwachen und Wiedereintreten in den Letharguszustand sind mit weitreichenden Änderungen verbunden, deren genaue zeitliche Abfolge aktuell analysiert wird.



ruhend



nach dem Aufwachen



(Nichols et al., 2017)

VB02: ein nicht-schlafendes Motorneuron
RIS: ein v.a. im Schlaf aktives Interneuron

Literaturverzeichnis

Choi, S., Chatzigeorgiou, M., Taylor, K.P., Schafer, W.R., and Kaplan, J.M. (2013). Analysis of NPR-1 Reveals a Circuit Mechanism for Behavioral Quiescence in *C. elegans*. *Neuron* 78, 869–880.

De Bono, M., and Bargmann, C.I. (1998). Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. *Cell* 94, 679–689.

Gray, J., Hill, J., and Bargmann, C. (2005). A circuit for navigation in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 102, 3184–3191.

Haspel, G., and O'Donovan, M.J. (2011). A perimotor framework reveals functional segmentation in the motoneuronal network controlling locomotion in *Caenorhabditis elegans*. *J Neurosci* 31, 14611–14623.

Hobert, O. (2003). Behavioral plasticity in *C. elegans*: paradigms, circuits, genes. *J Neurobiol* 54, 203–223.

Kim, D., Park, S., Mahadevan, L., and Shin, J.H. (2011). The shallow turn of a worm. *J Exp Biol* 214, 1554–1559.

Macosko, E., Pokala, N., Feinberg, E., Chalasani, S., Butcher, R., Clardy, J., and Bargmann, C. (2009). A hub-and-spoke circuit drives pheromone attraction and social behaviour in *C. elegans*. *Nature* 458, 1171–1175.

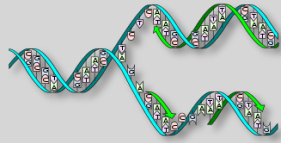
Nichols, A.L.A., Eichler, T., Latham, R., and Zimmer, M. (2017). A global brain state underlies *C. elegans* sleep behavior. *Science* 356.

Persson, A., Gross, E., Laurent, P., Busch, K.E., Bretes, H., and de Bono, M. (2009). Natural variation in a neural globin tunes oxygen sensing in wild *Caenorhabditis elegans*. *Nature* 458, 1030–1033.

Rogers, C., Reale, V., Kim, K., Chatwin, H., Li, C., Evans, P., and de Bono, M. (2003). Inhibition of *Caenorhabditis elegans* social feeding by FMRamide-related peptide activation of NPR-1. *Nat Neurosci* 6, 1178–1185.

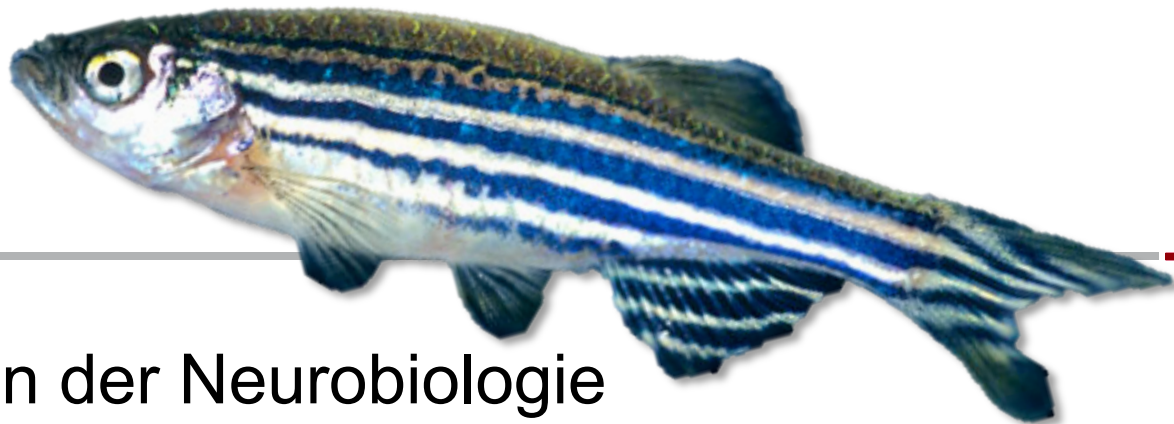
White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1976). The structure of the ventral nerve cord of *Caenorhabditis elegans*. *Philos Trans R Soc Lond B Biol Sci* 275, 327–348.

White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos Trans R Soc Lond B Biol Sci* 314, 1–340.



Diskussion

Genetische Modelle – von neuronalen Grundlagen zur klinischen Anwendung



UNI
FREIBURG

Modellsysteme in der Neurobiologie

Jochen Holzschuh

Universität Freiburg/Biologie 1

Abt. Entwicklungsbiologie

Was Sie erwartet



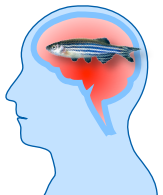
- Modellorganismus
Zebrafish



- Das Zebrafisch Gehirn



- Genom Editierung

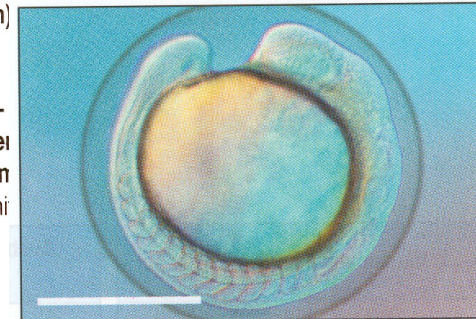
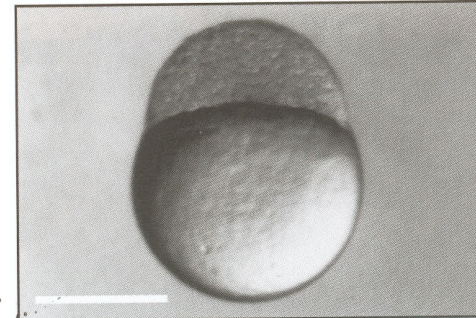
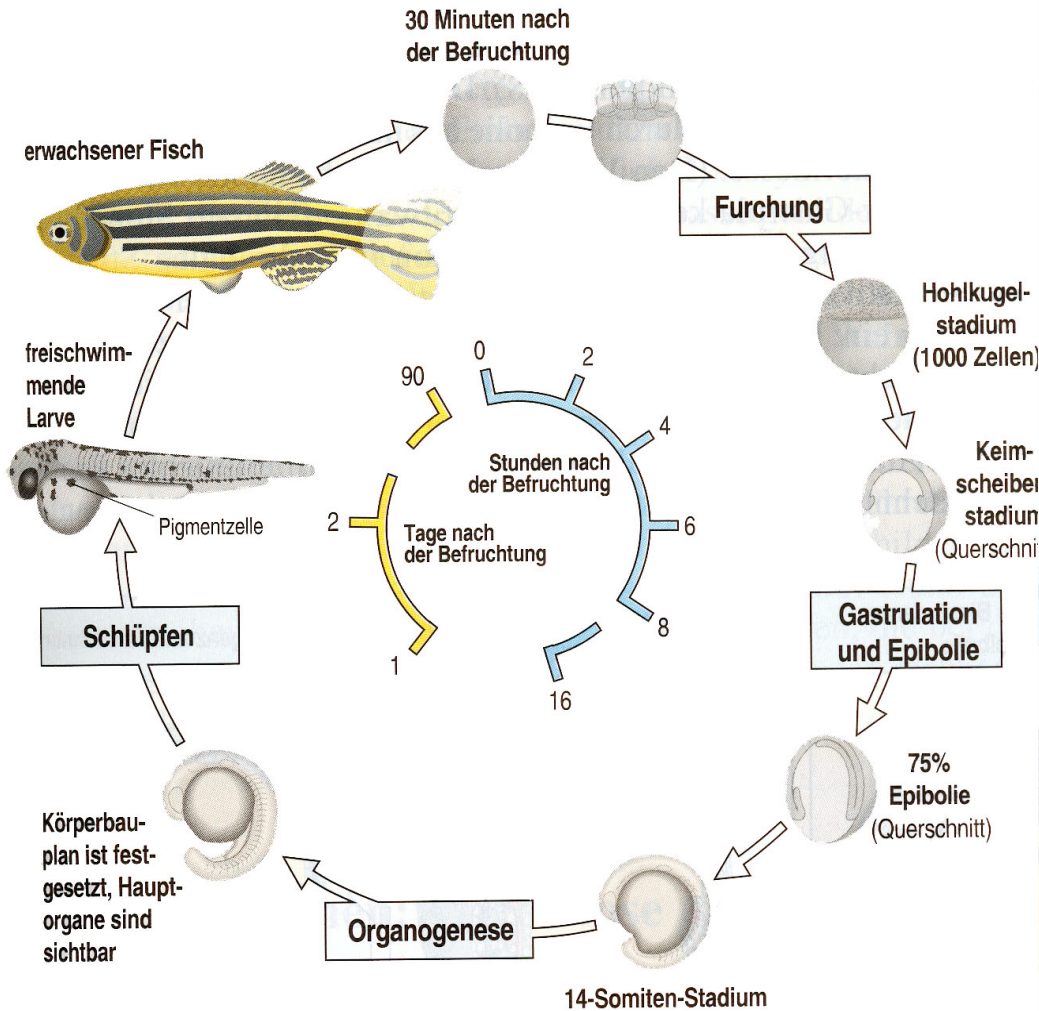


- Genetische Modelle im
Zebrafisch



Modelorganismus Zebrafisch

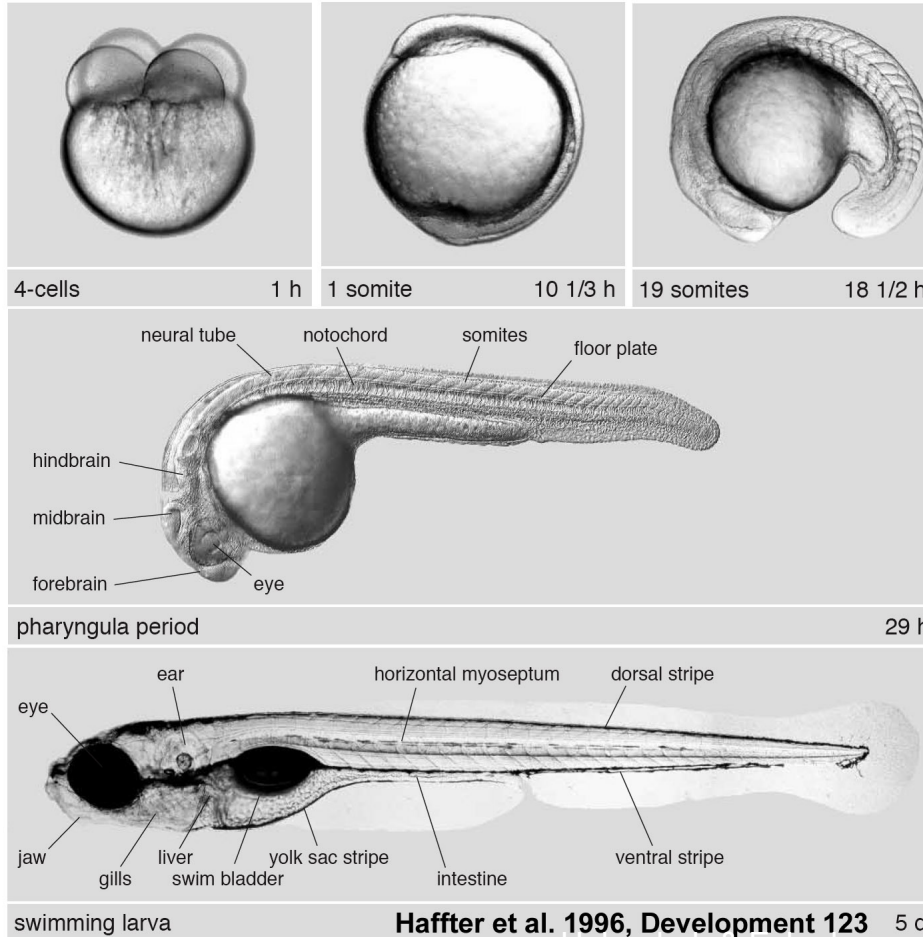
Danio rerio





Modelorganismus Zebrafisch

Danio rerio



■ Vorteile

- Embryologie

- Kleines leicht zu züchtendes Wirbeltier
- viele Nachkommen, die sich außerhalb der Mutter entwickeln
- Transparent: während der Entwicklung kann im lebenden Tier das Verhalten von Geweben oder einzelner Zellen verfolgt werden
- Regenerationsfähigkeit



Modelorganismus Zebrafisch

Danio rerio

- Einfache zelluläre und molekulare Manipulation
 - Einzelne Zellen oder ganze Zellverbände können entfernt oder in einen anderen Embryo transplantiert werden.
 - Überexpression eines Genprodukts durch RNA und DNA Injektionen
 - Gezielte Genomveränderungen durch TALEN oder Crispr Technologien
- Genom vollständig sequenziert
- Großer Bestand an mutanten und transgenen Linien
-



Modelorganismus Zebrafisch

Danio rerio

- Große Ähnlichkeit mit dem Menschen
 - Etwa 71% der menschlichen Gene besitzen mindestens ein Ortholog im Zebrafisch
 - Über 80 Prozent der bislang bekannten Gene, die beim Menschen zu Krankheiten führen können, finden sich auch im Zebrafisch.
 - Gene zwischen Zebrafischen und Säugern/Mensch sind hoch konserviert

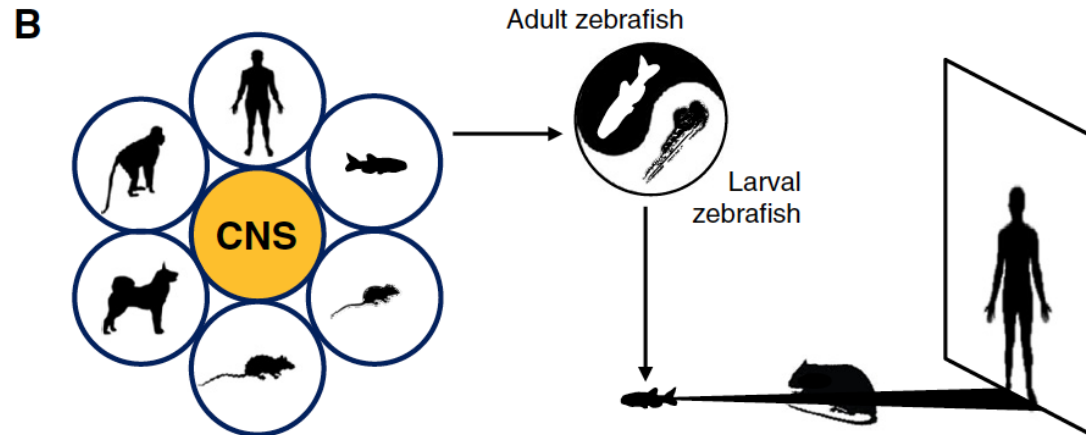
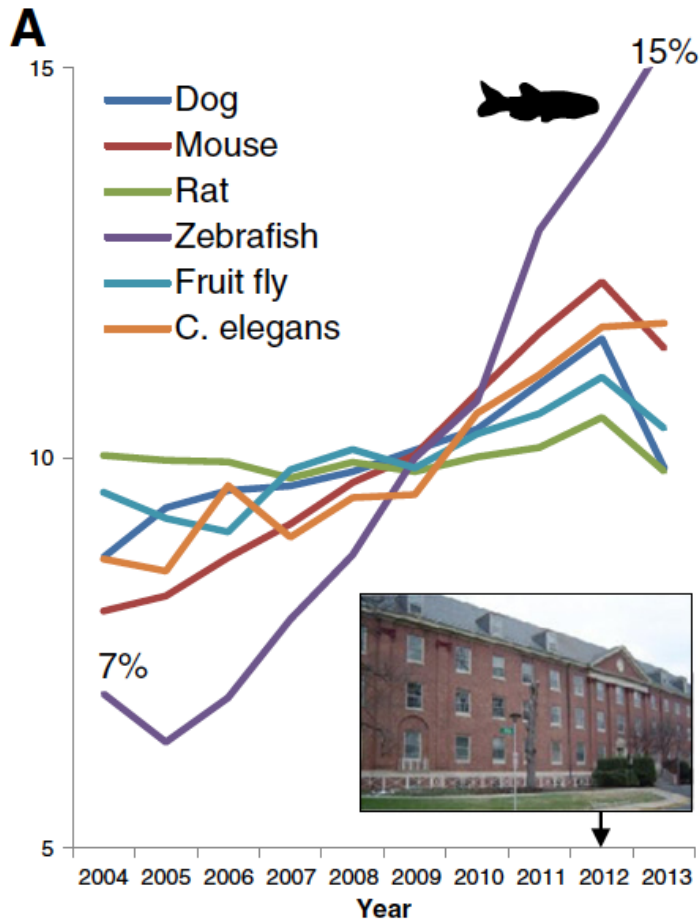
Zur Untersuchung von Menschlichen Erkrankungen die durch Gendefekte ausgelöst werden gilt der Zebrafisch daher als ein geeigneter Modellorganismus.



Modelorganismus Zebrafisch

Danio rerio

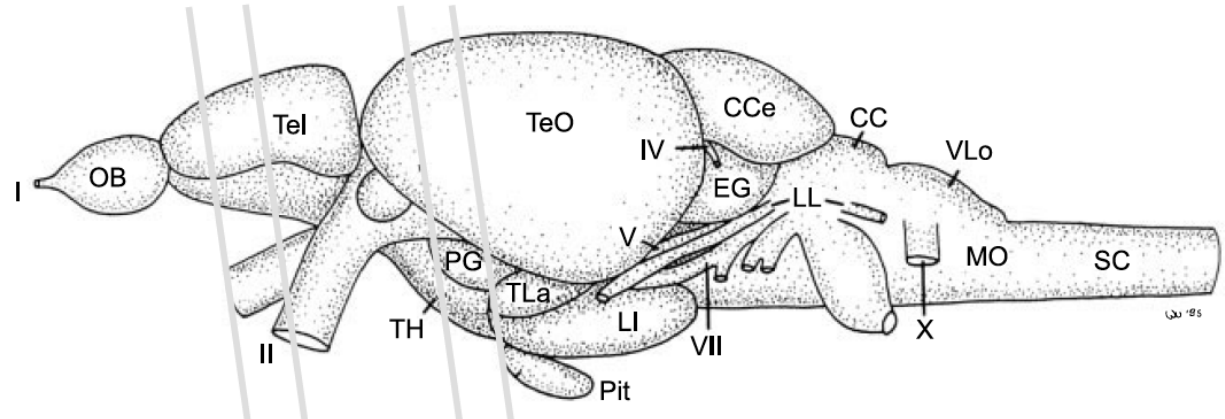
Der wachsende Nutzen von Zebrafisch-Modellen in der Biomedizin



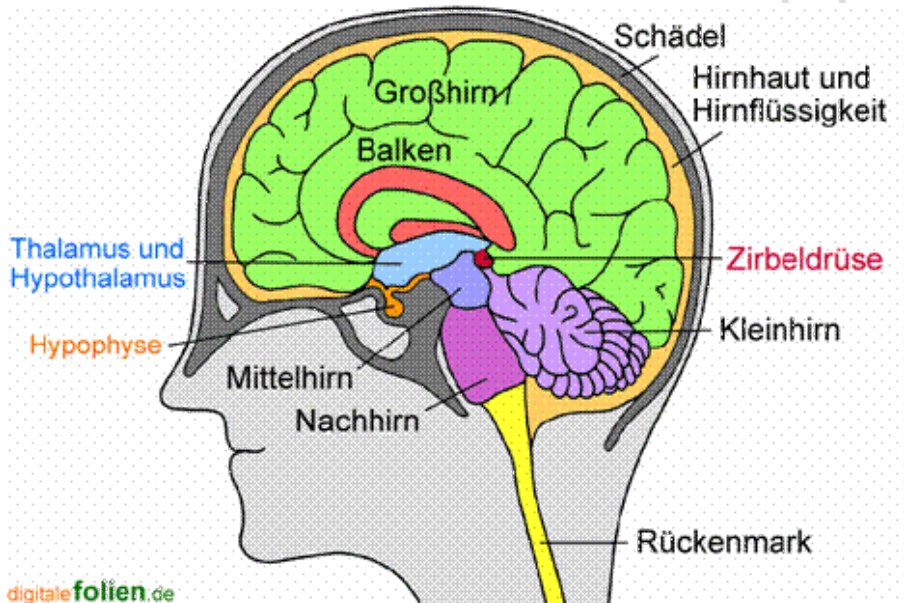
Kalueff et al. 2014



Das Zebrafisch Gehirn



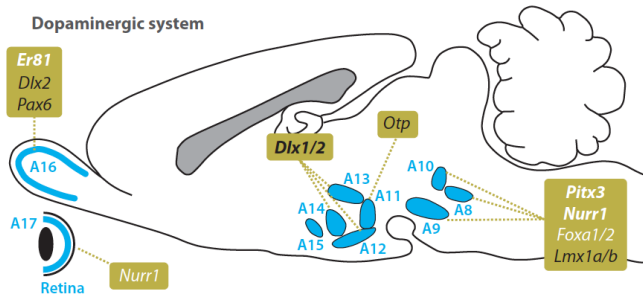
Wullimann und Müller 2004



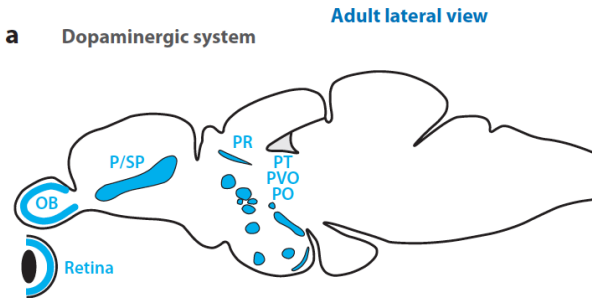


Vergleich zwischen Neurotransmittersystemen im Säuger und Zebrafisch

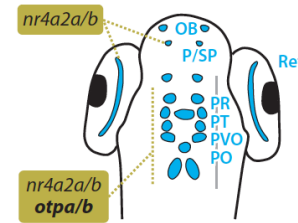
a Dopaminergic system



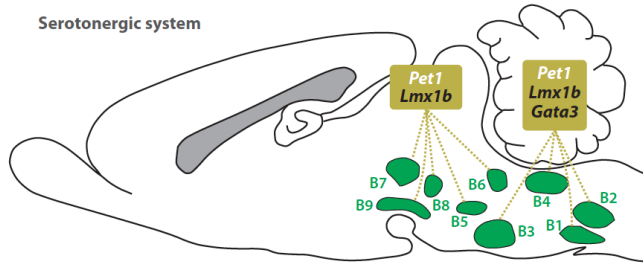
a Dopaminergic system



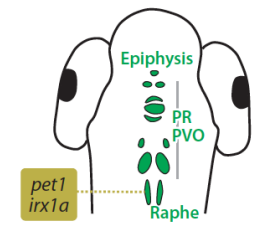
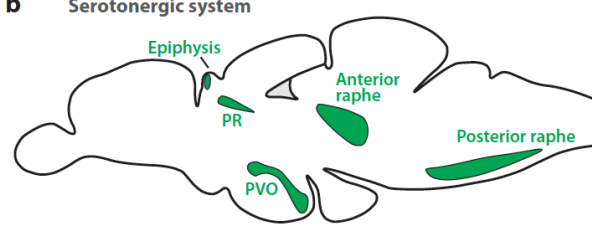
6-day-old dorsal view



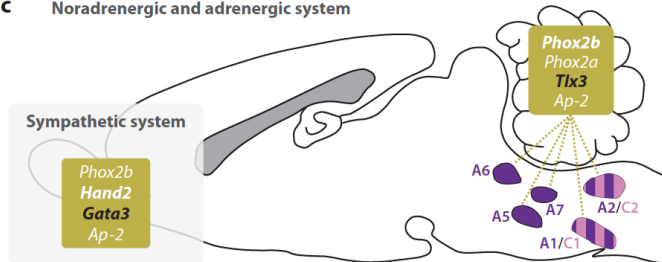
b Serotonergic system



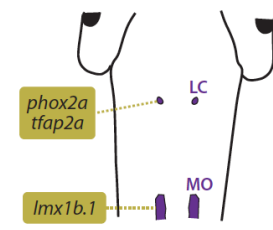
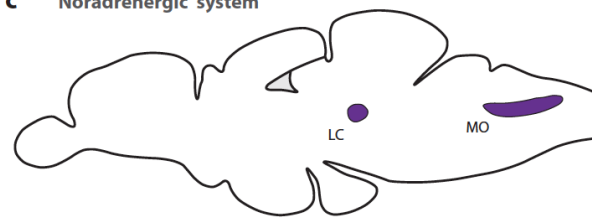
b Serotonergic system



c Noradrenergic and adrenergic system



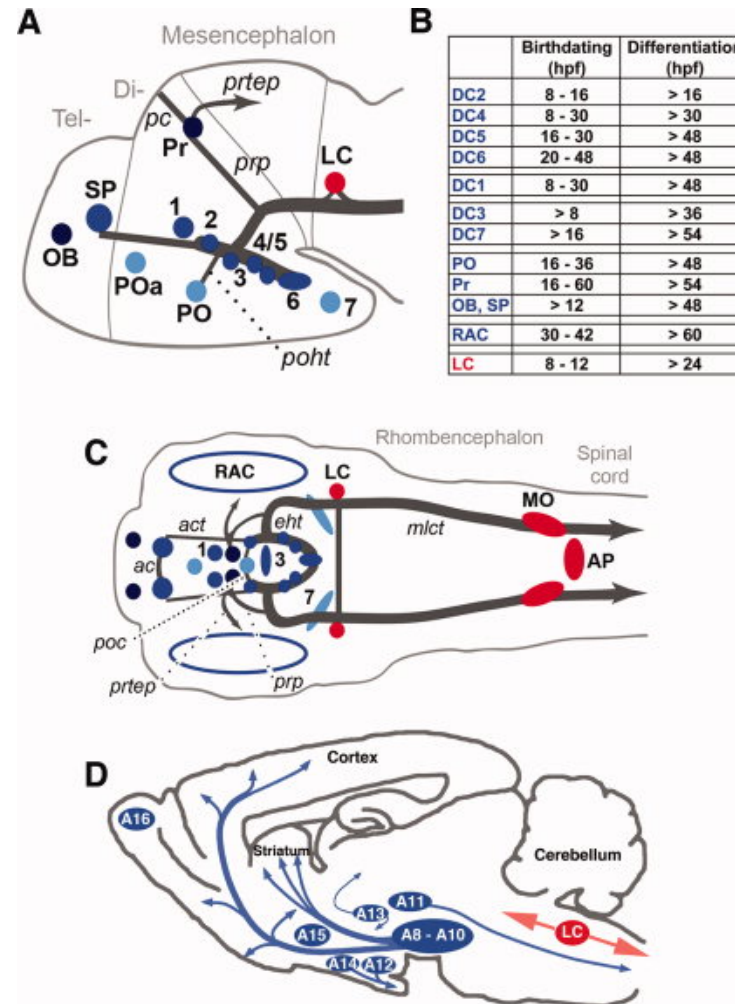
c Noradrenergic system



Flames & Hobert, 2011



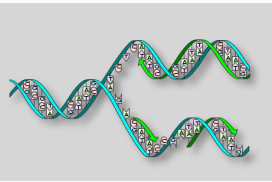
Vergleich zwischen Neurotransmittersystemen im Säuger und Zebrafisch



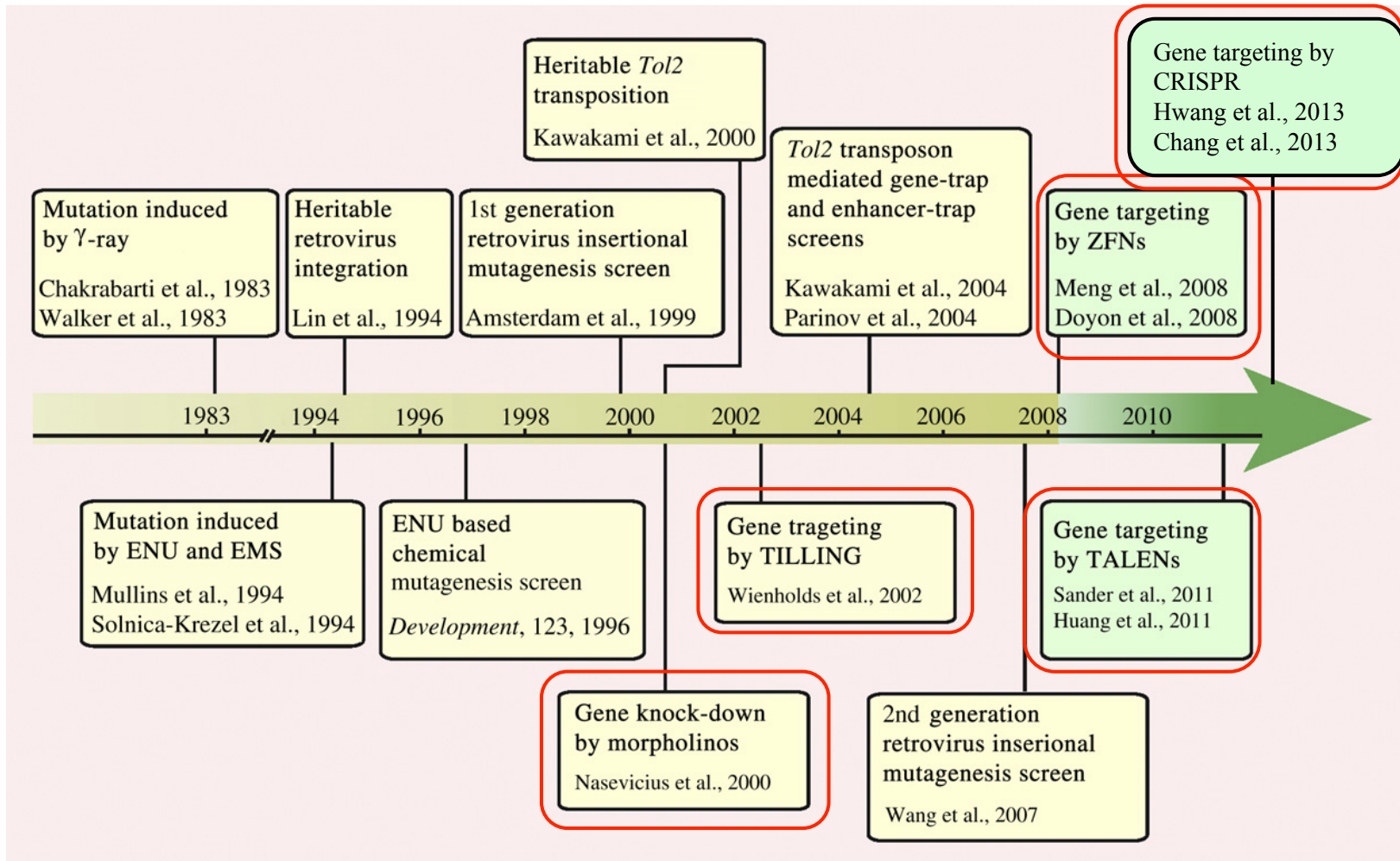
Developmental Neurobiology

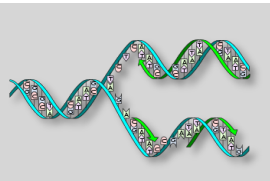
Volume 72, Issue 3, pages 256-268, 10 FEB 2012 DOI: 10.1002/dneu.20911

<http://onlinelibrary.wiley.com/doi/10.1002/dneu.20911/full#fig1>

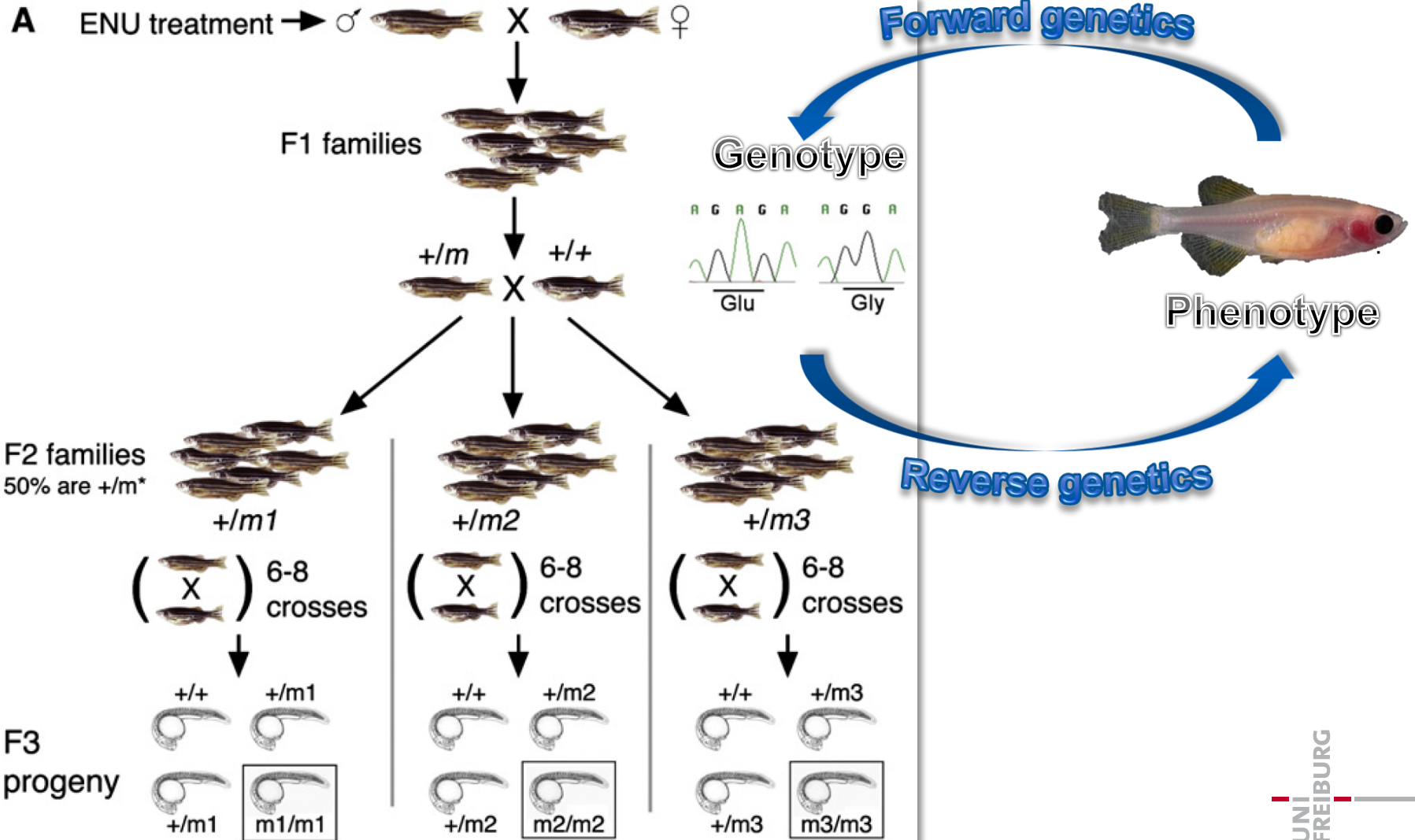


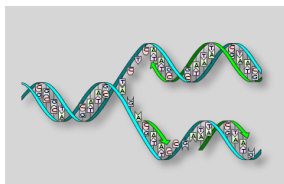
Ein historischer Überblick auf die „genetischen Tool“ im Zebrafisch





Vorwärts gerichtete Genetik: Mutagenese Screens





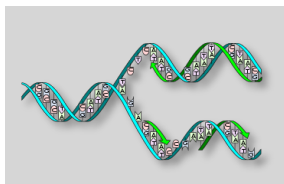
Rückwärts gerichtete Genetik: “Gene silencing“

Post-transkriptionales “Gene silencing“:

- RNA interference
- morpholino antisense oligos

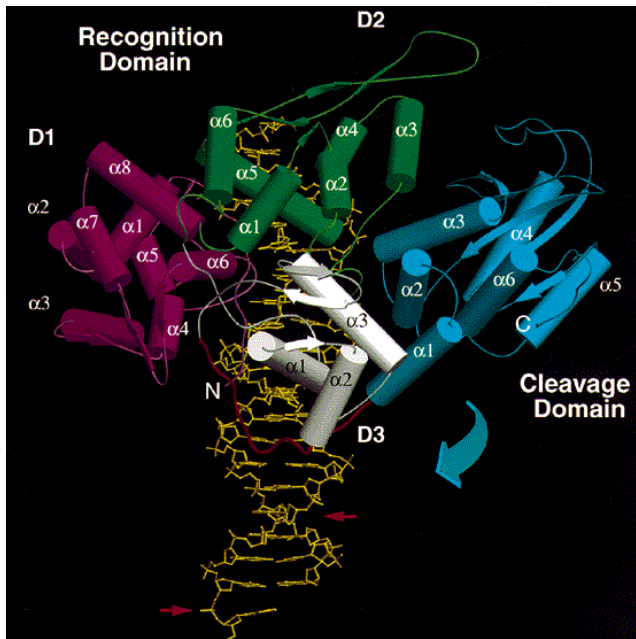
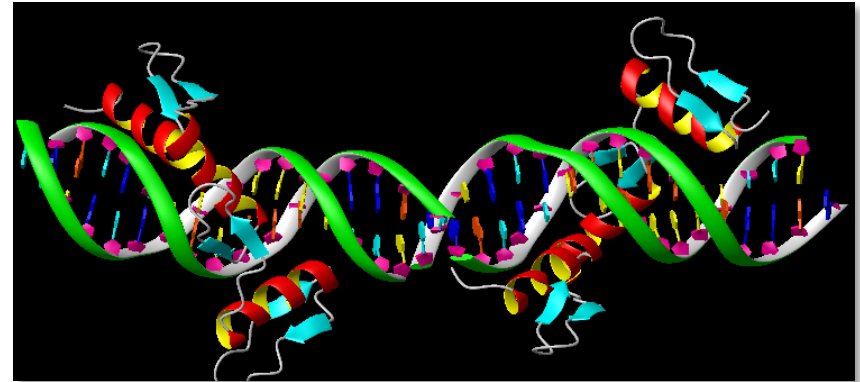
Transkriptionelles “Gene Silencing“:

- Homologe Rekombination “knock-out” in Embryonalenstammzellen
- ZFNs (**Z**inkfinger **N**ukleasen)
- TALENs (**T**ranscription-**a**ctivator-like **E**ffektor **N**ukleasen)
- CRISPRs

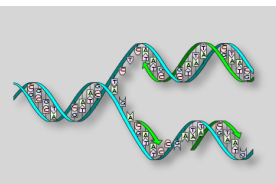


Genome Editierung mit gentechnisch veränderten Zinc Finger Nukleasen

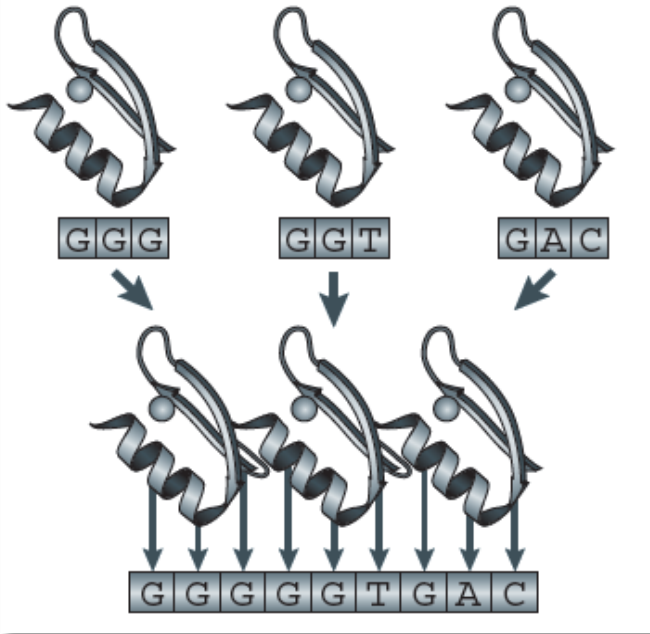
Zinkfingerdomänen treten typischerweise als Tandemwiederholungen mit zwei, drei oder mehr Fingern auf, die die DNA-bindende Domäne des Proteins umfassen. Diese können sequenzspezifisch auf DNA binden.



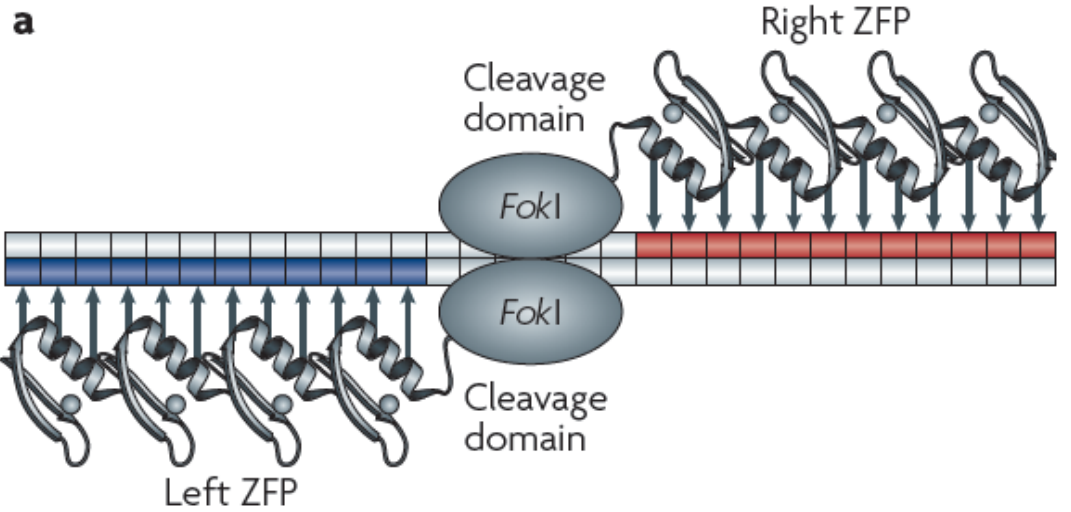
FokI NuKlease: Ist eine bakterielle Restriktionsendonuklease Aus dem *Flavobacterium okeanokoites*. Sie besteh aus einer N-terminalen DNA-bindenden Domäne und einer unspezifischen DNA-Spaltungsdomäne am C-Terminus.



Struktur und Design von Zink Finger Nucleases



Urnov et al., 2010



FokI: unspezifischen DNA-Spaltungsdomäne

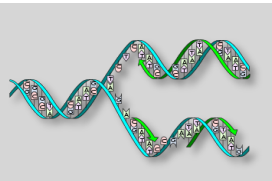
Genome Editierung mit TALE

Transcription activator-like effectors

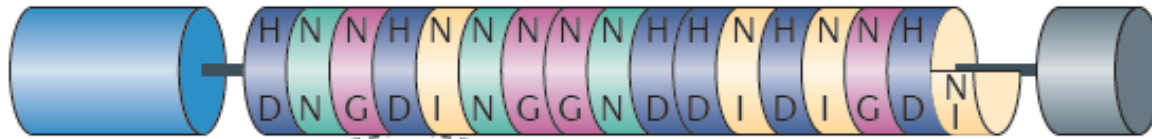
TALE sind bakterielle Proteine der pathogenen Gattung *Xanthomonas*. Sie werden durch das Bakterium in die Pflanzenzelle injiziert und wirken dort im Zellkern als Transkriptionsaktivatoren



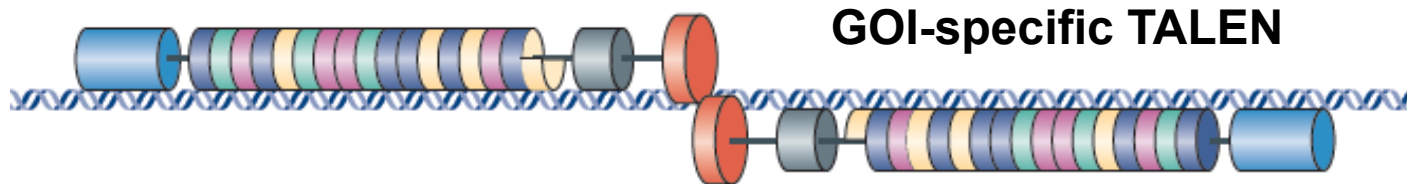
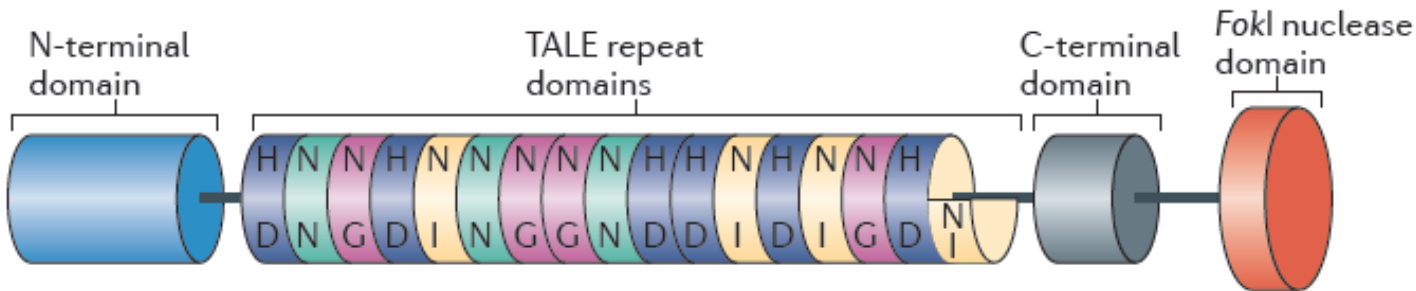
Voytas & Joung, 2009 and Boch & Bonas, 2010



TALE Nukleasen (TALEN)

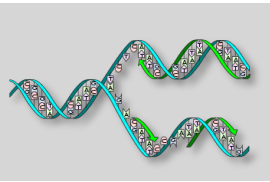


LTPDQVVAIAS **HD**GGKQALETVQRLLPVLCQDHG

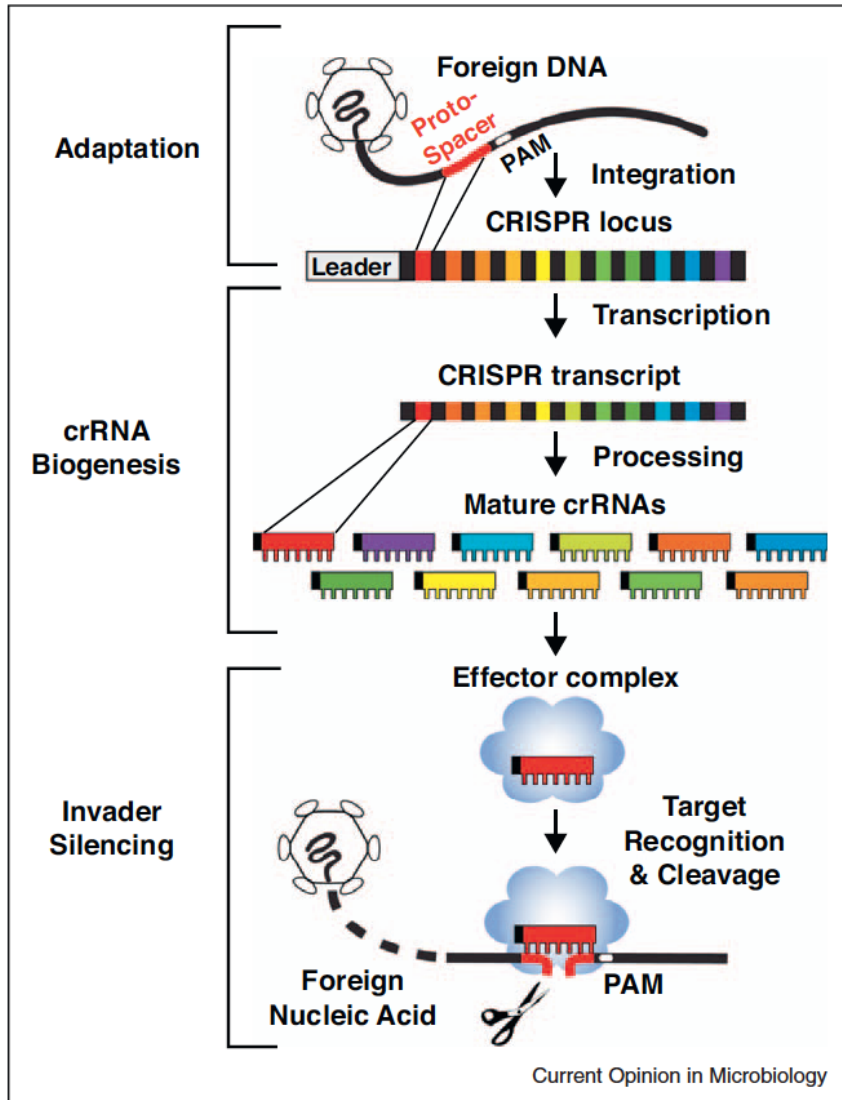


GOI-specific TALEN

Joung & Sander, NatRevMCB, 2013

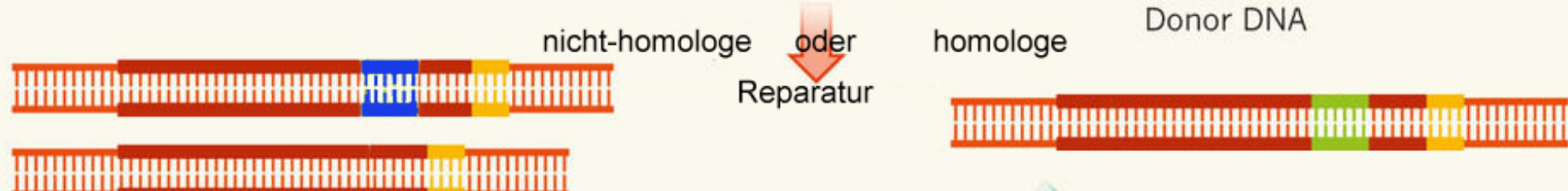
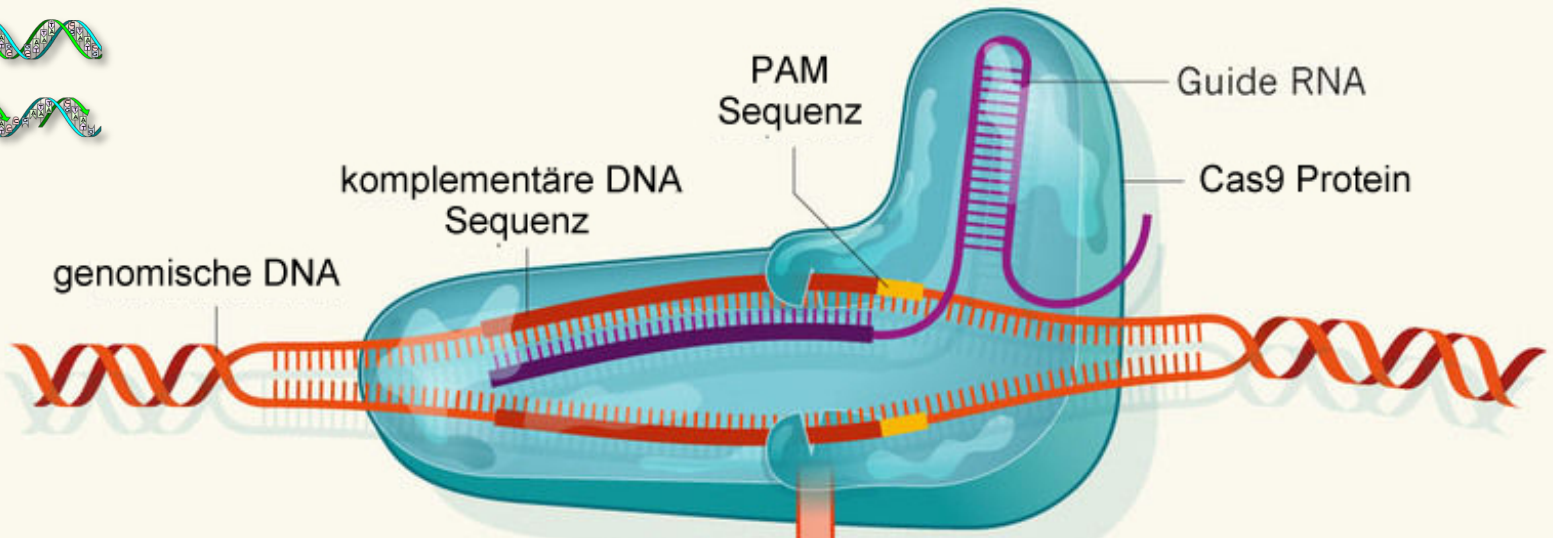
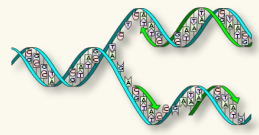


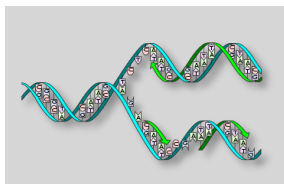
Genom Editierung durch CRISPR Cas Systeme



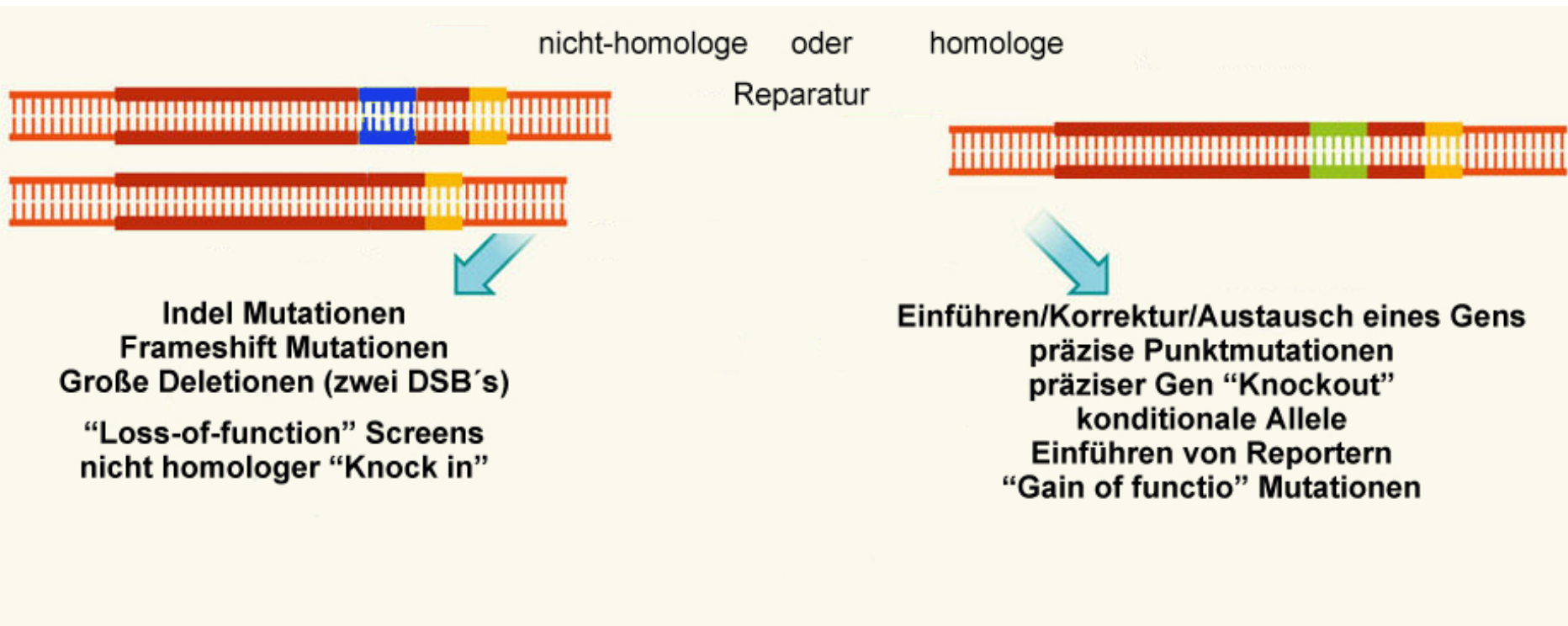
CRISPR Clustered Regularly Interspaced Short Palindromic Repeat

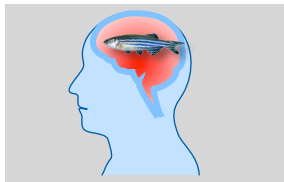
Kleine RNA-basierte
Abwehrsysteme - Immunität
gegen Viren, Plasmide und
andere mobile genetische
Elemente in Archaea und
Bakterien





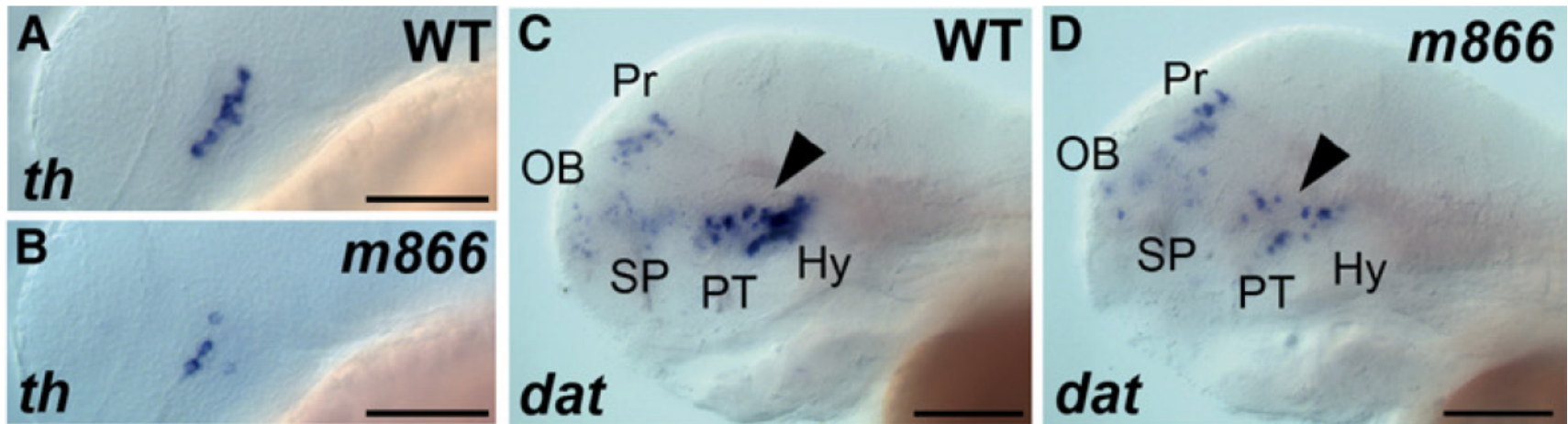
Genom Editierung durch CRISPR Cas Systeme



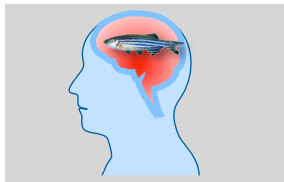


Entdeckung von Genen die für die Gehirnentwicklung benötigt werden

- Der Transkriptionsfaktor *Otpa* wird für die Entwicklung bestimmter (A11) dopaminergener Neurone benötigt. Im Zebrafisch...

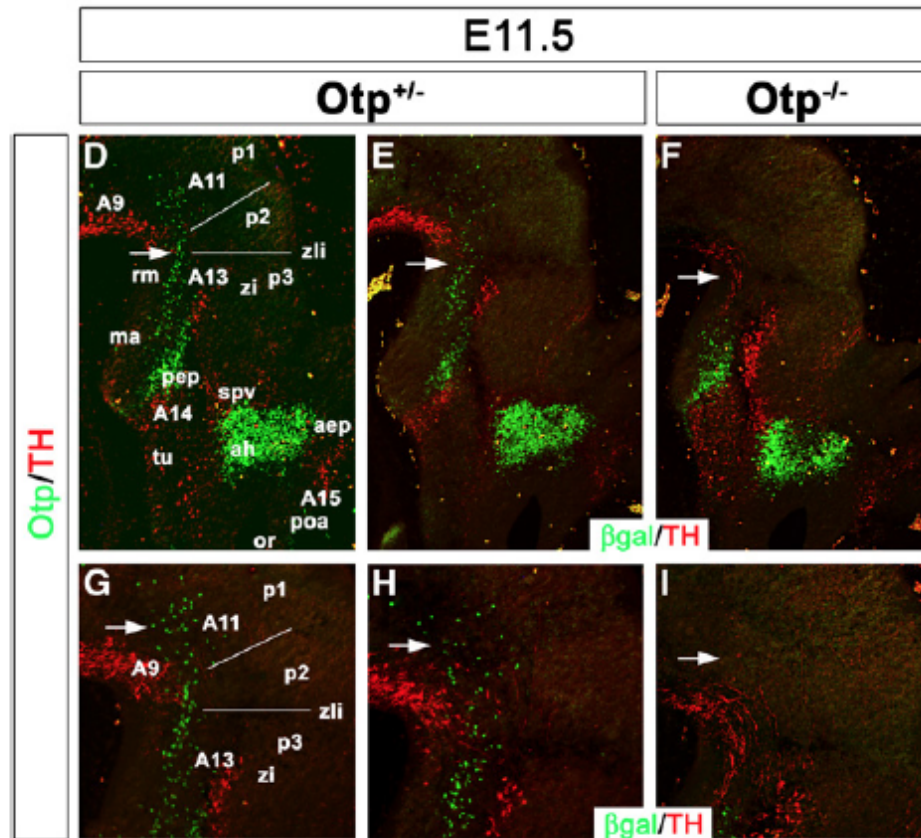


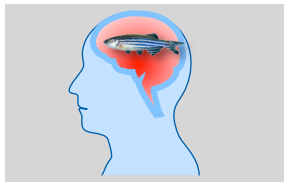
Ryu et al., 2007



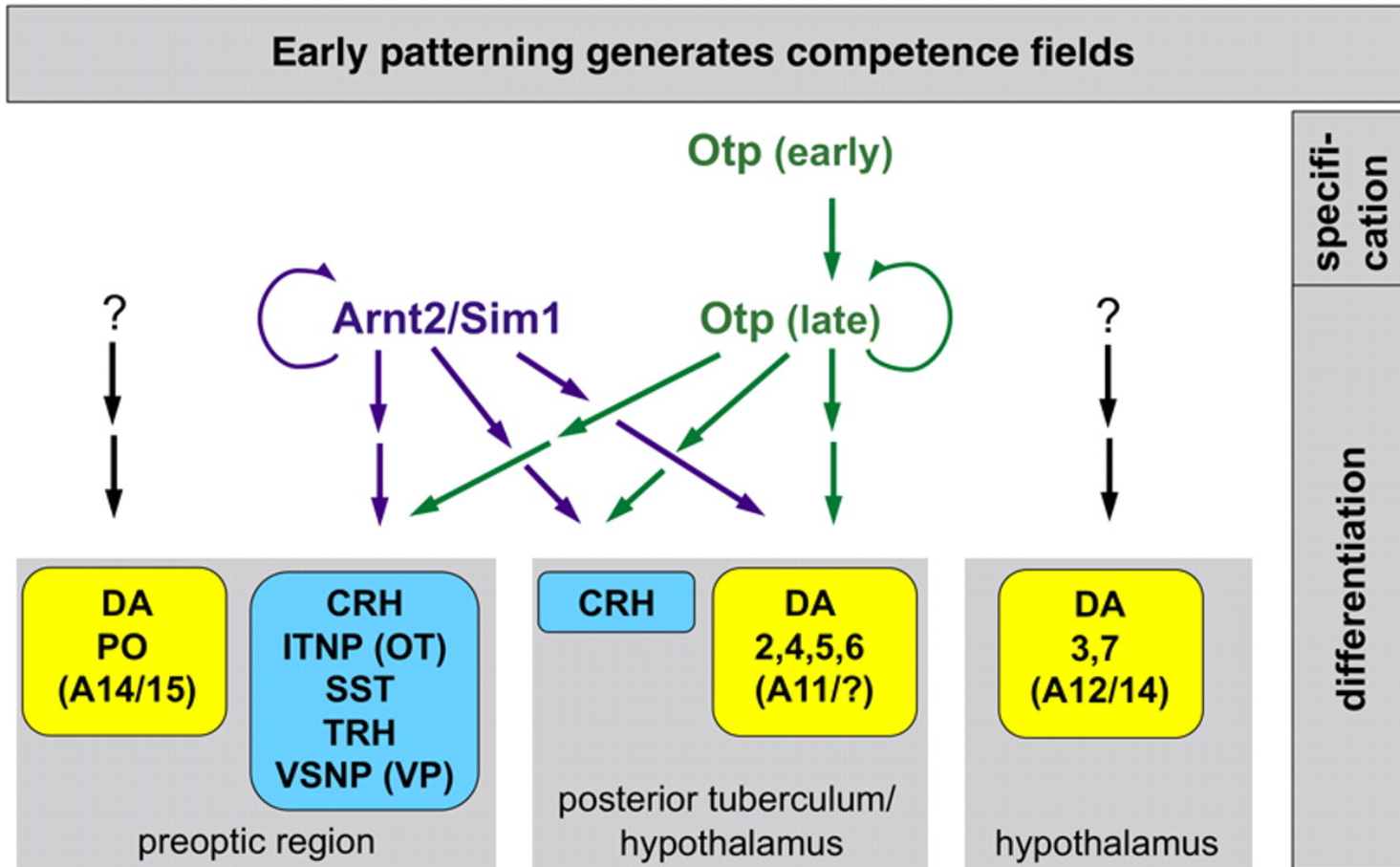
Entdeckung von Genen die für die Gehirnentwicklung benötigt werden

-und in der Maus

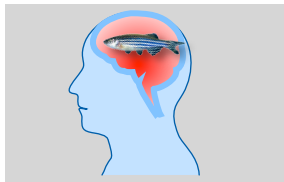




Entdeckung von Genen die für die Gehirnentwicklung benötigt werden

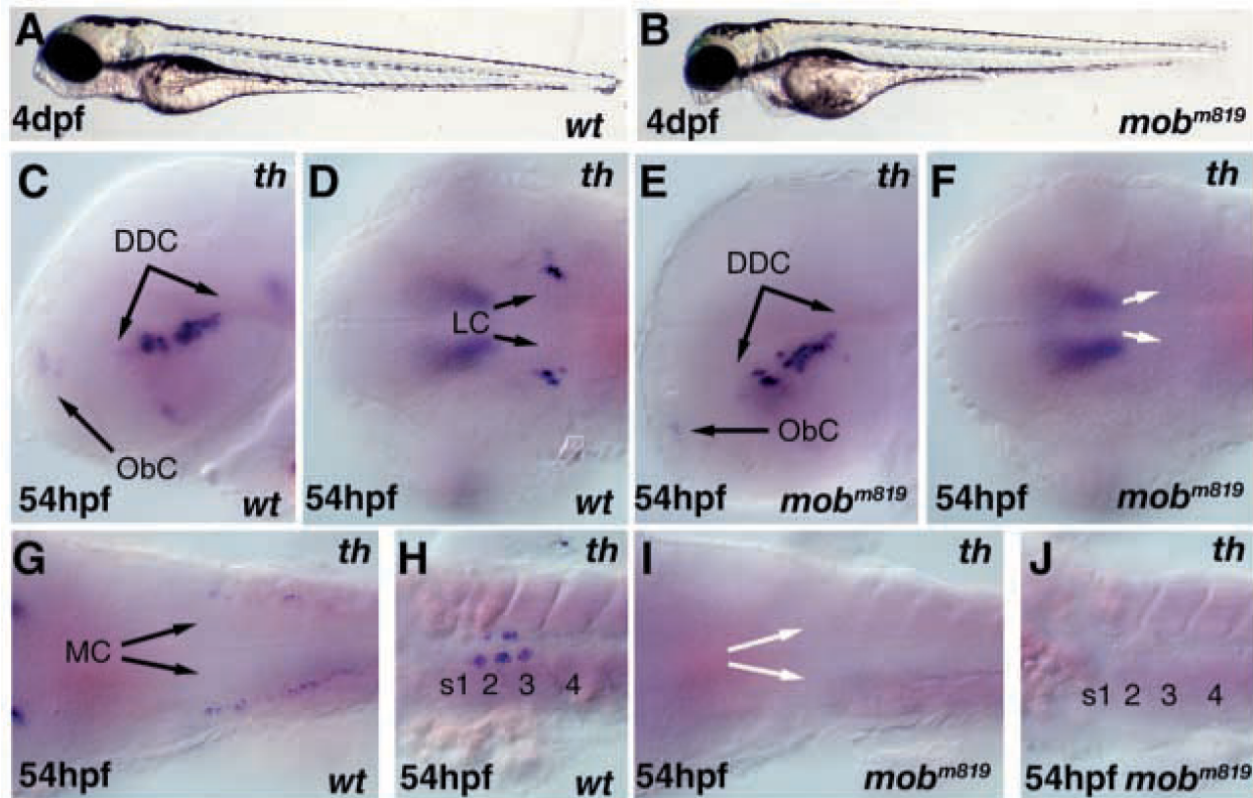


Heiko Löhr et al. Development 2009;136:1007-1017

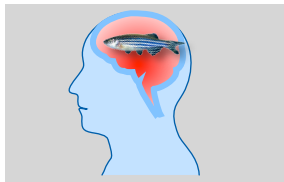


Entdeckung von Genen die für die Gehirnentwicklung benötigt werden

- Der Transkriptionsfaktor Tfp2 α wird für die Entwicklung aller Noradrenergen Neurone benötigt.

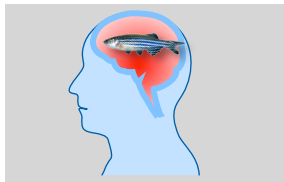


Holzschuh et al., 2003



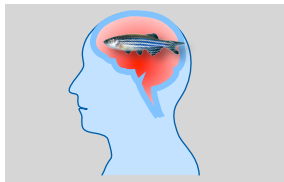
Spektrum autistischer Störungen (ASS) *Autism spectrum disorder (ASD)*

- **Drei Hauptmerkmale**
 - **gestörte soziale Interaktion**
 - **beeinträchtigte Kommunikation und Sprache**
 - **wiederholte, stereotype Verhaltensweisen und Interessen**
- **Vererbbar**
- **Polygenetisch**
 - **mehr als 500 Gene können 20% der ASS Fälle erklären**

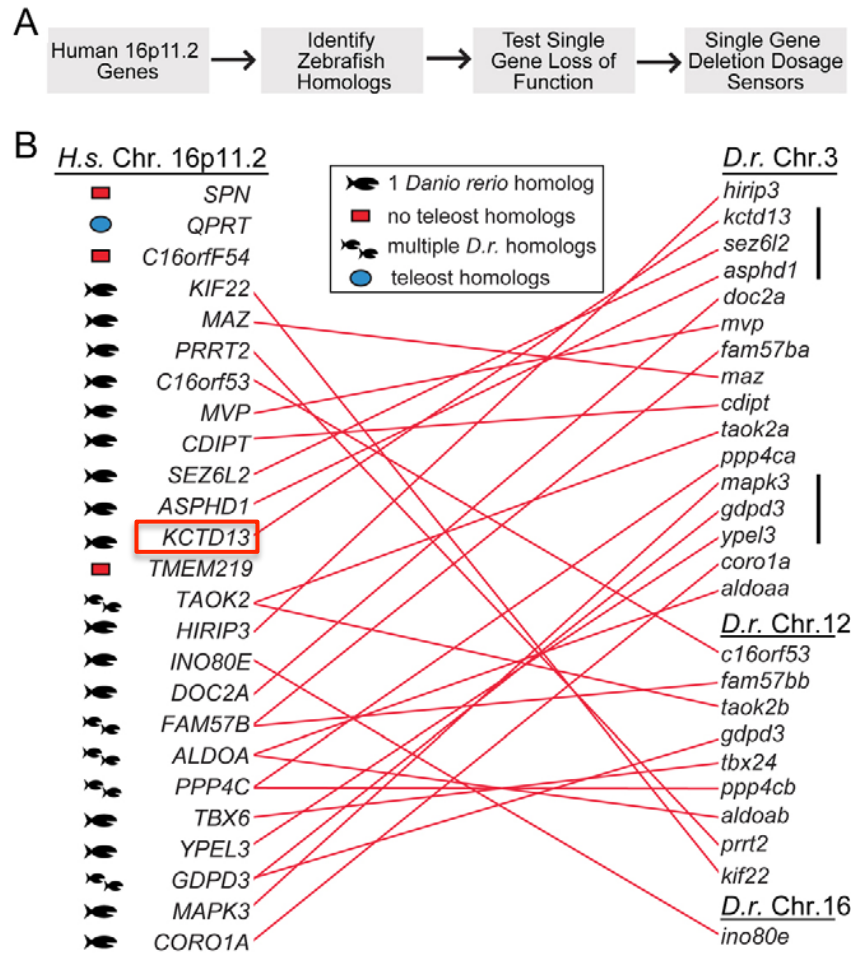


Kopienzahlvariation der 16p11.2 Region führt zu ASS

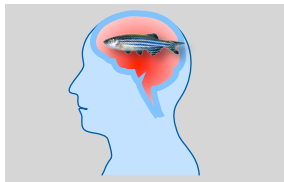
- Eine Deletion auf 16p11.2 (29 Gene) wurde mit Makrocephalie, Epilepsie, Autismus und ASS assoziiert
- Eine Vervielfältigung der Region wird mit Autismus, Schizophrenie und Mikrocephalie assoziiert.



Die meisten der Gene auf 16p11.2 finden sich auch im Zebrafisch



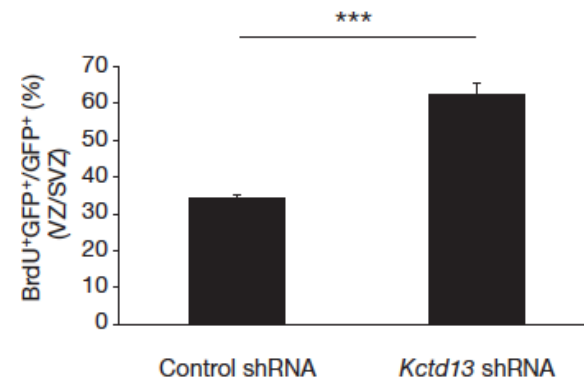
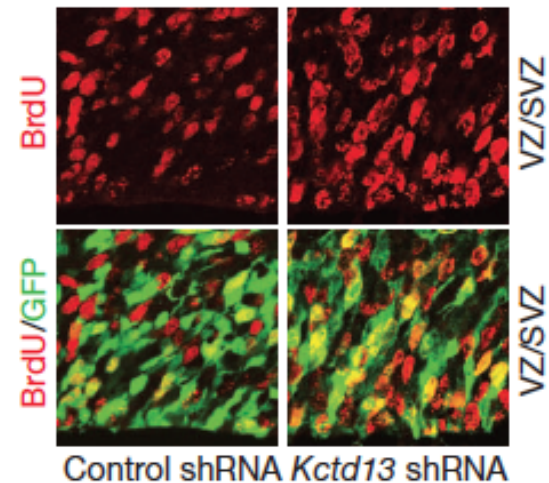
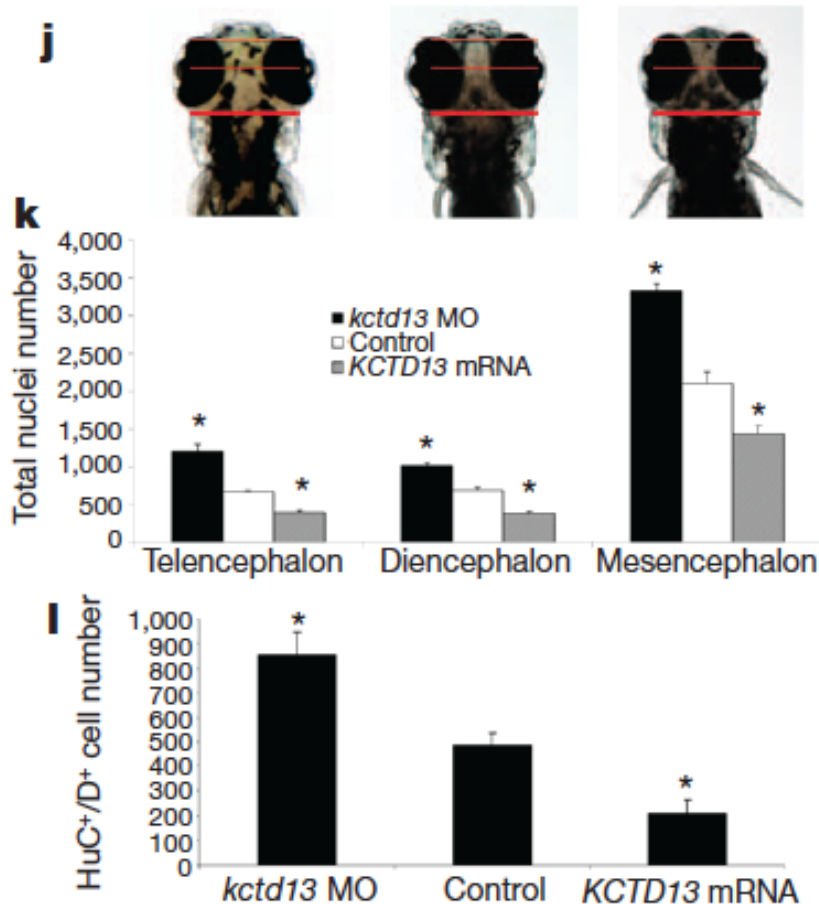
Alicia Blaker-Lee et al. *Dis. Model. Mech.* 2012;5:834-851

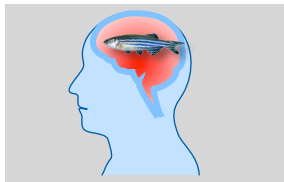


KCTD13 Kopienzahlvariation im Genetischen Modell

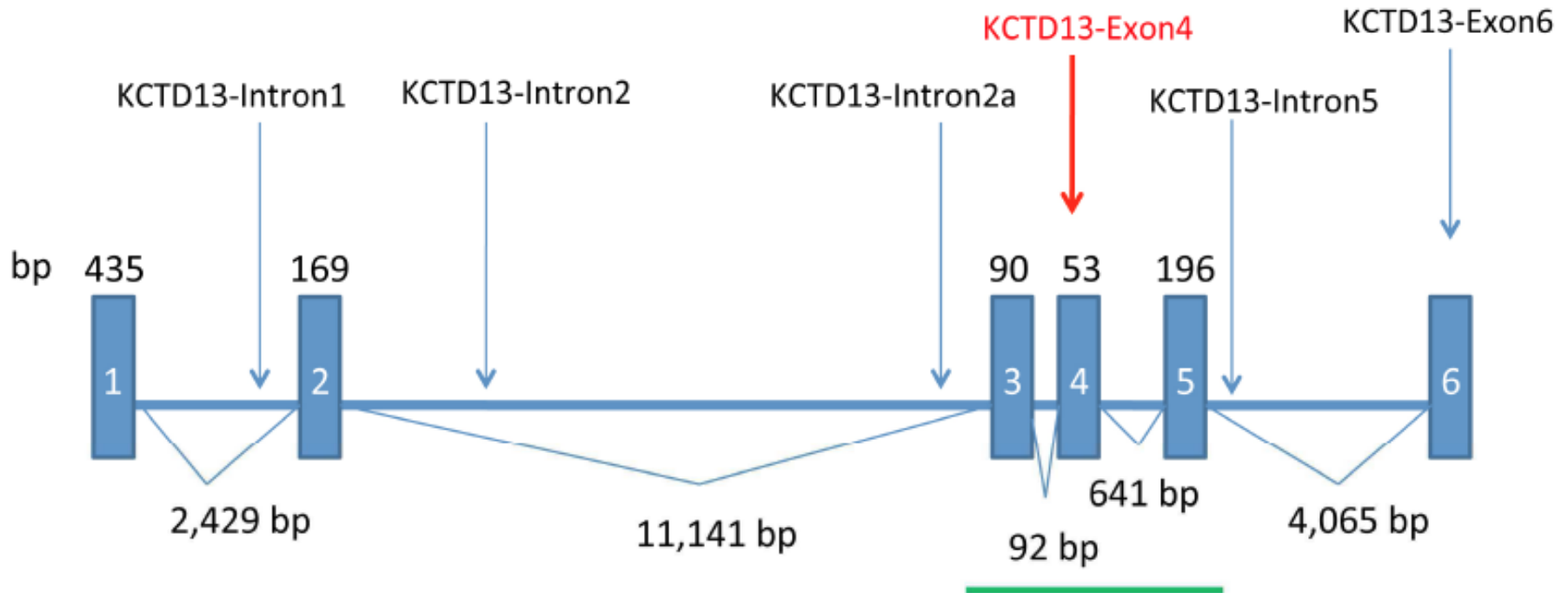
Zebrafisch

Maus

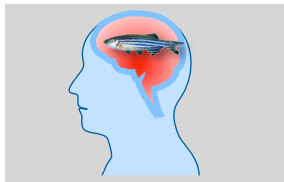




KCTD13 Kopienzahlvariation in einem Autismus Patienten

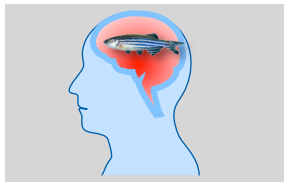


Deletion von Exon 4



CNTNPA2 ein Risiko Gene für ASS

- **Contactin Associated Protein-like 2**
 - Zelladhesions Molekül, das spannungsabhängige Kalium Kanäle in bestimmten axonalen Region lokalisiert
 - Homozygote Funktionsverlustmutationen sind stark mit ASS gekoppelt
 - Zebrafische besitzen zwei *cntnap2* Gene (Paraloge)



Genetisches Modell für die CNTNPA2 Funktion

- ZNF induzierte Zebrafisch Mutanten in *cntnap2a* und *b* wurden generiert um die Auswirkungen des Funktionsverlust auf die Entwicklung/Funktion des Gehirns zu studieren und einen Medikamenten Screen durchzuführen

Wild Type Cntnap2a (1316 aa), Cntnap2b (1315 aa)



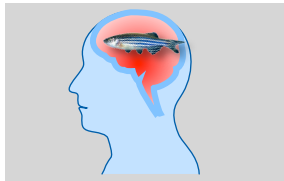
Cntnap2a Δ 121 (120 aa), Δ 25 (152 aa)



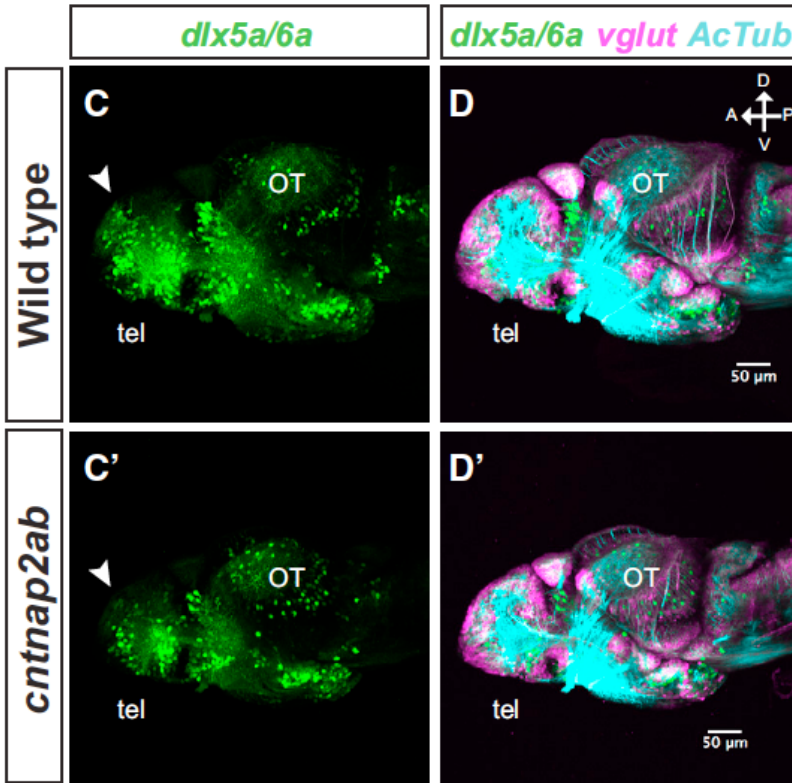
Cntnap2b 31ins (142 aa), Δ 7 (60 aa)



Hoffman et al., (2015,Neuron)

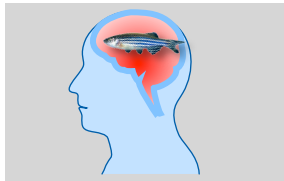


cntnpa2 Verlust führt zu einer Reduzierung inhibitorischer Neurone

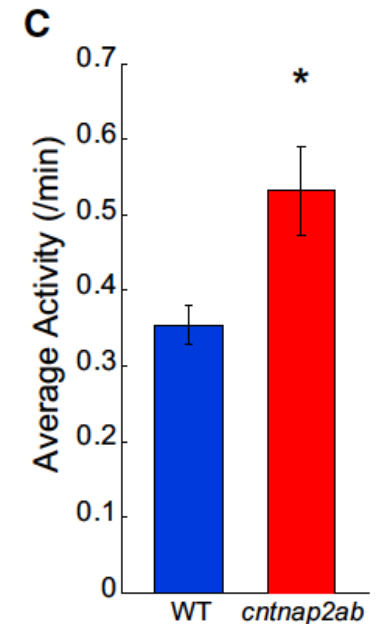
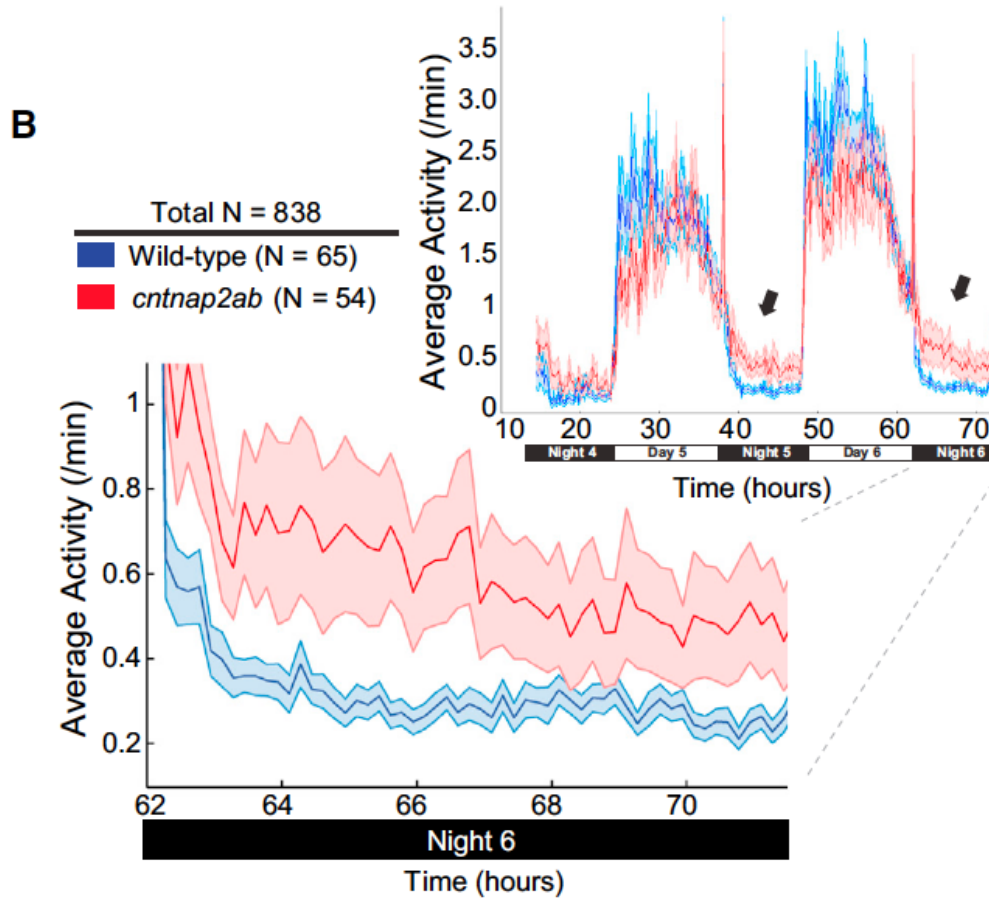
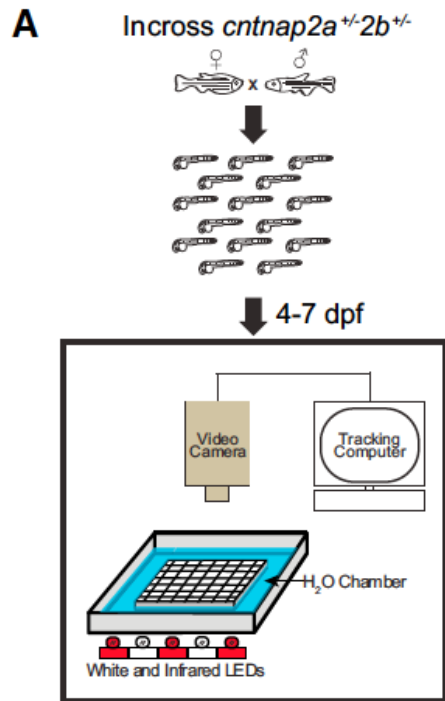


dlx5a/6a = GABA Neurone inhib.
vglut = Glutamaterge Neurone exit.

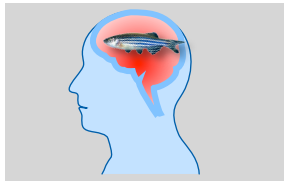
Hoffman et al., (2015,Neuron)



cntnpa2 Verlust führt zu einer nächtlichen Hyperaktivität

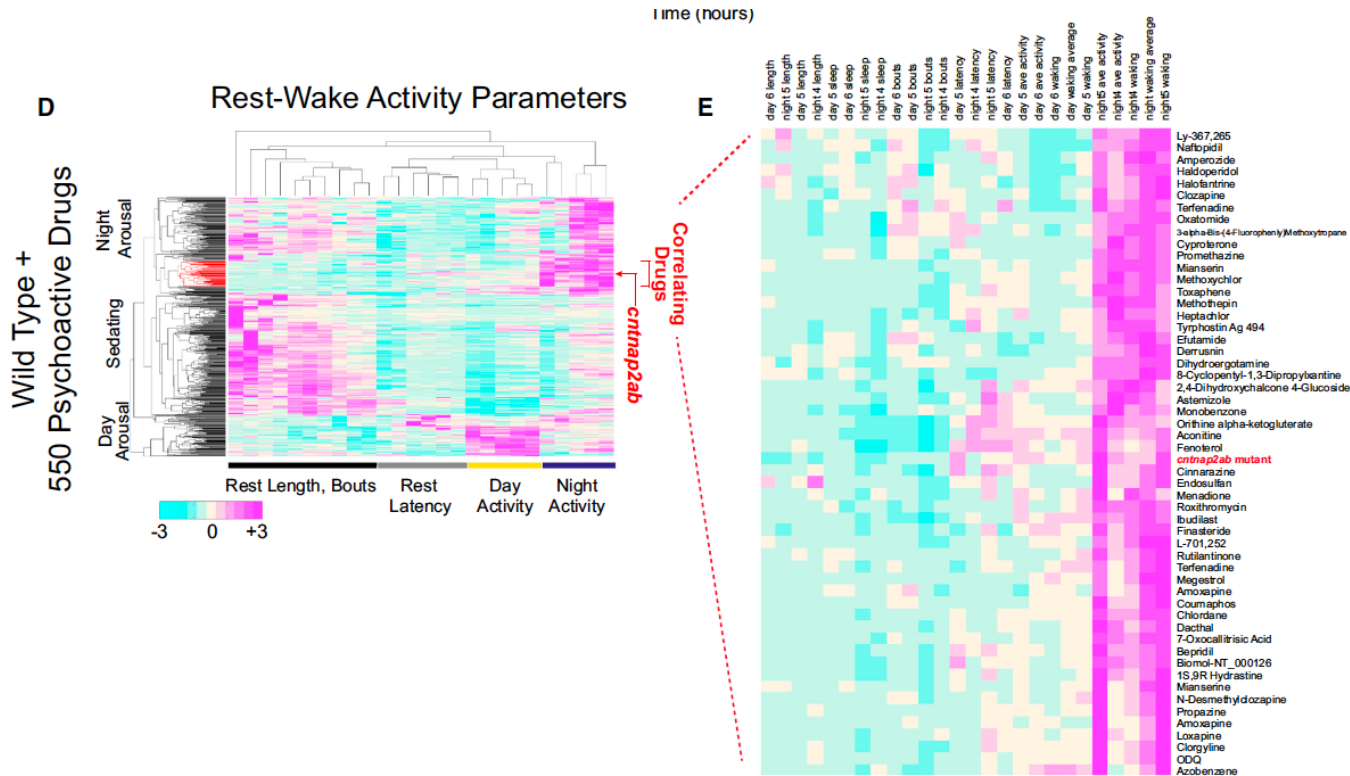


Hoffman et al., (2015,Neuron)

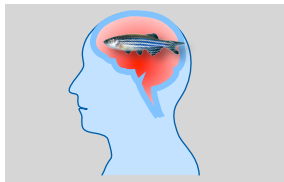


cntnpa2 Verlust führt zu einer nächtlichen Hyperaktivität

Der Verhaltensfingerabdruck der *cntnap2ab*-Mutante wurde mit einem Datensatz der Verhaltensprofile von Wildtyp-Larven, die 550 psychoaktiven Verbindungen ausgesetzt waren verglichen (Rihel et al., 2010).



Hoffman et al., (2015,Neuron)

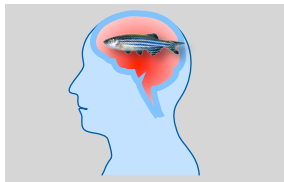


Verhaltensreaktionen von *cntnpa2*-Mutanten zu psychoaktiven Mitteln

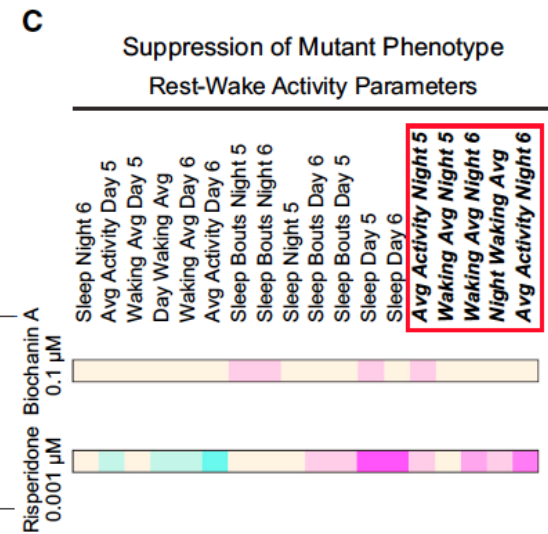
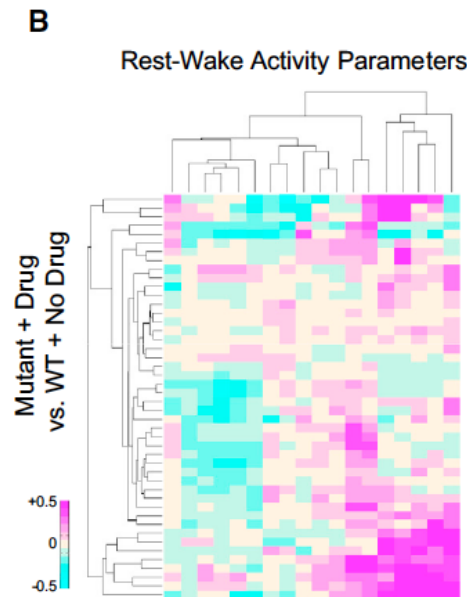
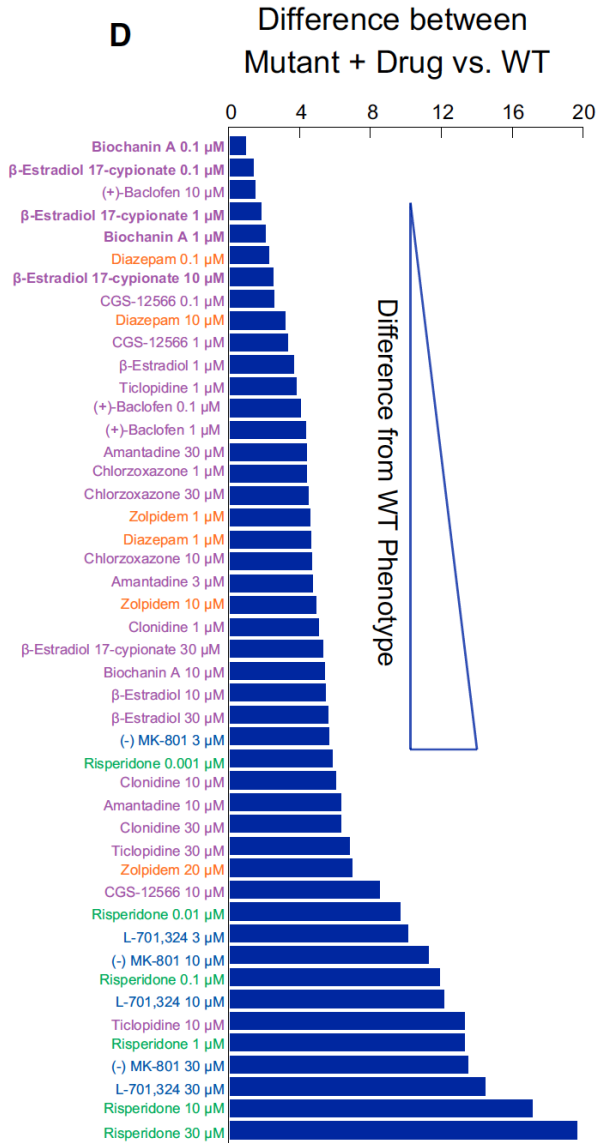
A Psychoactive Drugs Tested

Compound Name	Biological Target(s)	Rationale for Selection
L-701,324	NMDA glycine site antagonist	Correlating
(-)-MK-801	Non-competitive NMDA receptor antagonist	Correlating
Amantadine	Dopaminergic; increases dopamine synthesis/release, inhibits reuptake	Anti-Correlating
(+)-Baclofen	GABA-B receptor agonist	Anti-Correlating
β -Estradiol	Estrogen receptor- β agonist	Anti-Correlating
β -Estradiol 17-cypionate	Estrogen receptor- β agonist	Anti-Correlating
Biochanin A	Estrogen receptor- β agonist	Anti-Correlating
CGS-12066	5HT1B serotonin receptor agonist	Anti-Correlating
Chlorzoxazone	Centrally acting muscle relaxant	Anti-Correlating
Clonidine	α 2-adrenergic receptor agonist	Anti-Correlating
Ticlopidine	Platelet aggregation inhibitor	Anti-Correlating
Risperidone	Dopamine (D2) and serotonin (5-HT2A) receptor antagonist	FDA-approved for irritability in ASD
Zolpidem	Nonbenzodiazepine GABA-A receptor agonist	GABAergic deficit
Diazepam	Benzodiazepine; positive allosteric GABA-A receptor modulator	GABAergic deficit

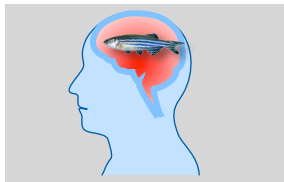
Hoffman et al., (2015,Neuron)



Verhaltensreaktionen von *cntnpa2*-Mutanten zu psychoaktiven Mitteln

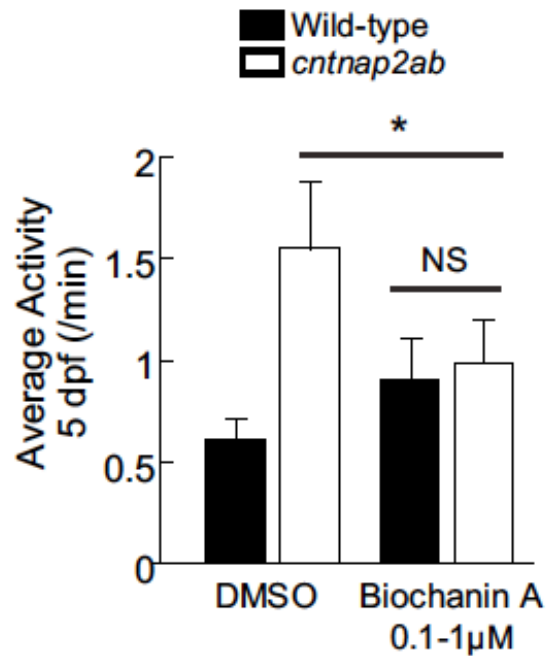


Hoffman et al., (2015, Neuron)

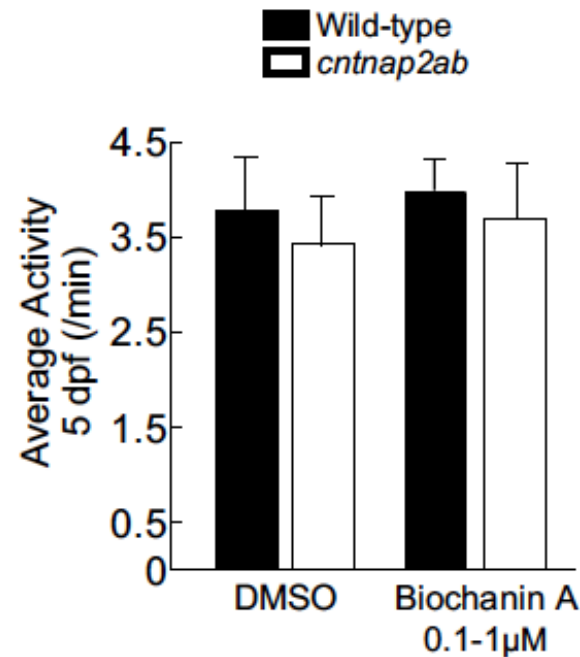


Biochanin A rettet den Verhaltens Phänotyp von *cntnpa2* Mutanten

G Nighttime Activity



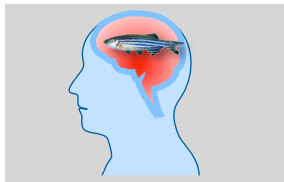
H Daytime Activity



Hoffman et al., (2015,Neuron)

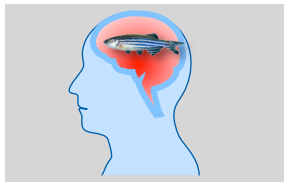
Biochanin A ist ein Phytoöstrogen das schwache östrogenartige biologische Aktivität aufweist.

Es findet sich in bestimmten Hülsenfrüchten (z.B Rotklee)



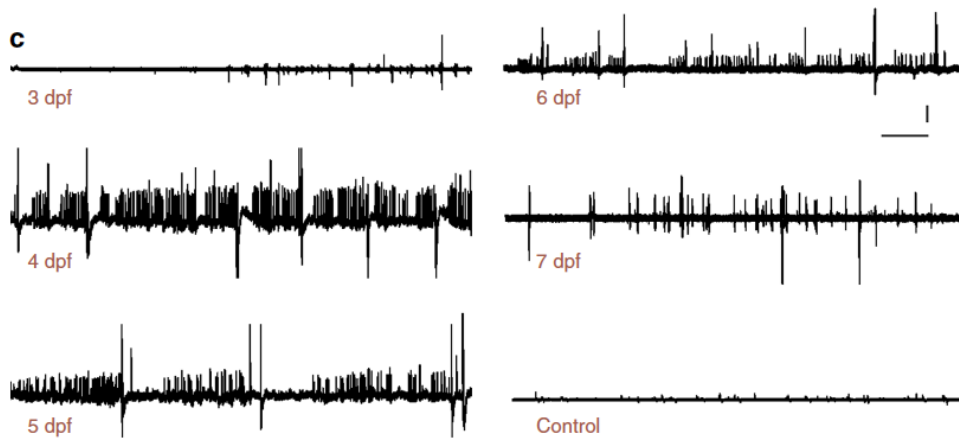
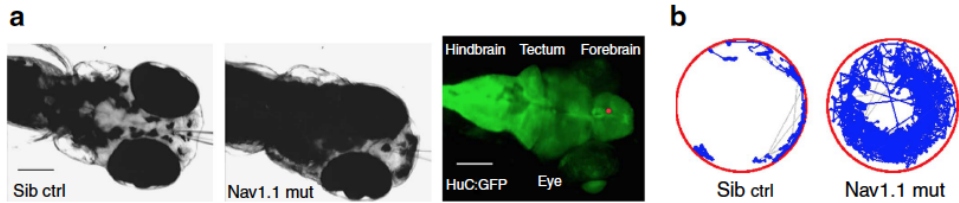
Epilepsie

- Unter den genetisch bedingten Epilepsien, wurden mehr als 650 Varianten im *SCN1A*-Gen identifiziert (kodiert für einen Spannungsabhängiger Natrium Kanal)



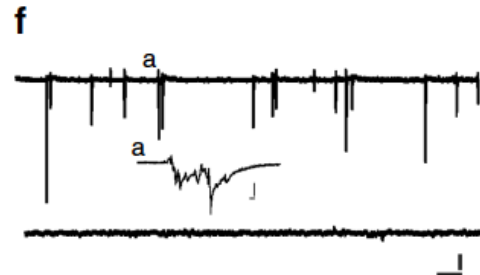
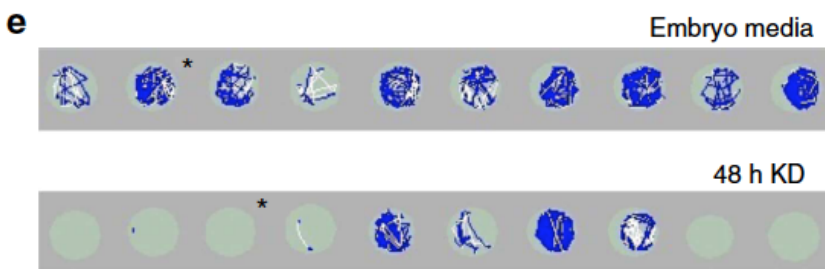
Epilepsie

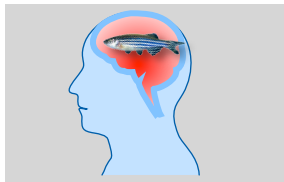
scn1Lab Modell im Zebrafisch



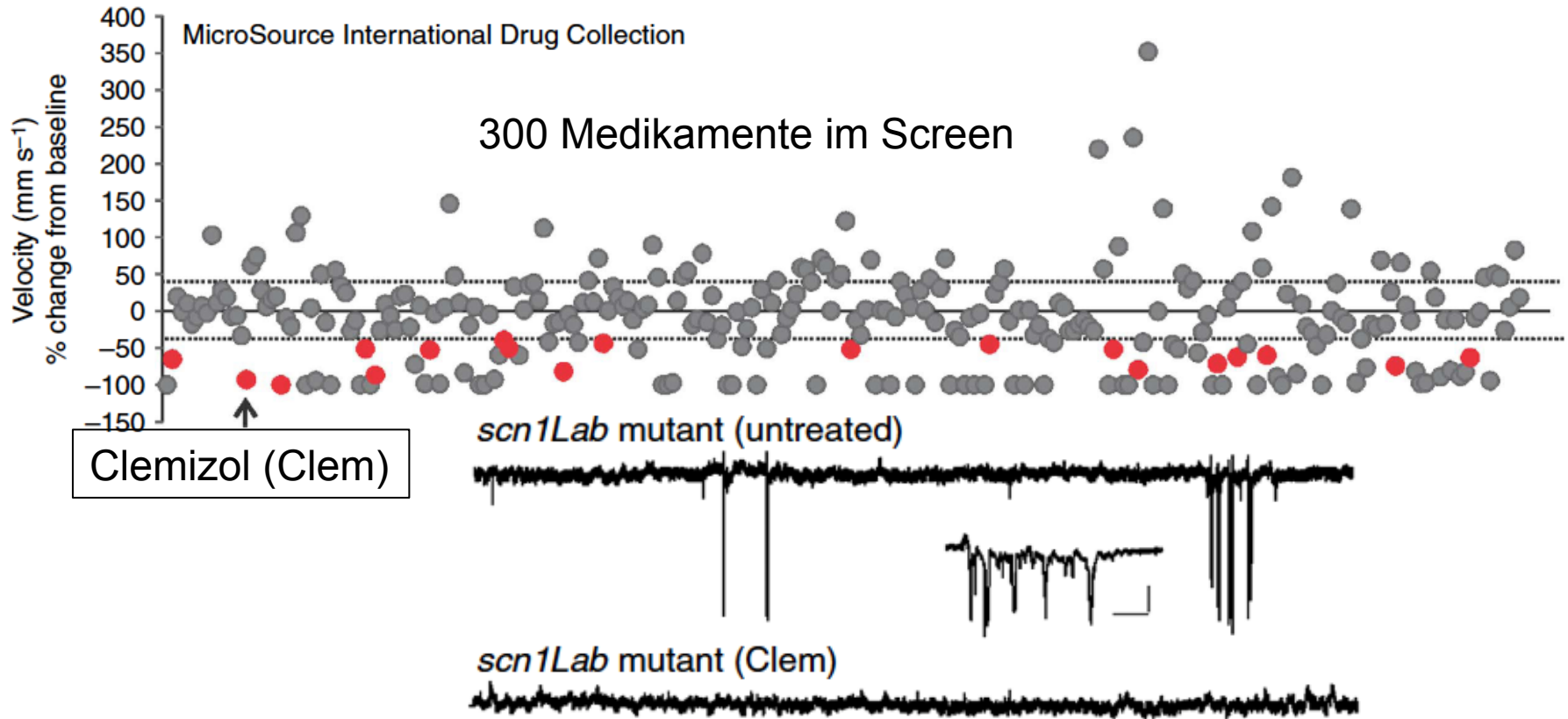
Spontane Krampfanfälle in *scn1Lab* Mutanten

Nach ketogener Diät reduzieren sich die spontanen Krampfanfälle in *scn1Lab* Mutanten



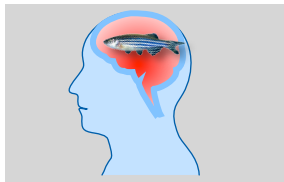


Medikamenten Screen in *scn1Lab* Mutanten



Baraban et al., 2013

Das anfallshemmende Clemizol ist eigentlich ein Antiallergikum (Antihistaminikum, entdeckt in 1950s)



Lorcaserin unterdrückt Krampfanfälle in *scn1Lab* Mutanten und Dravet Syndrom Patienten

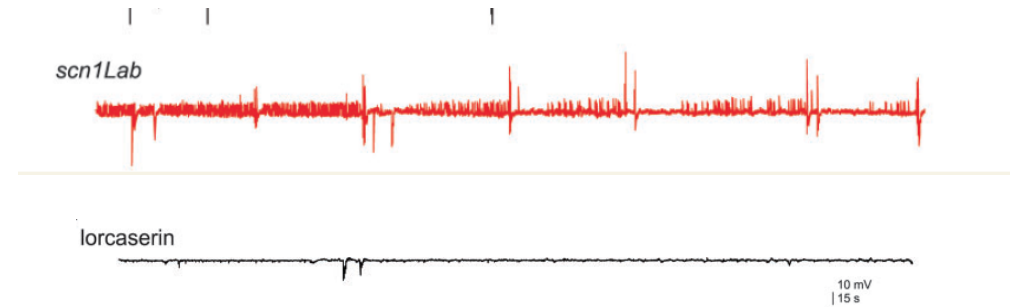
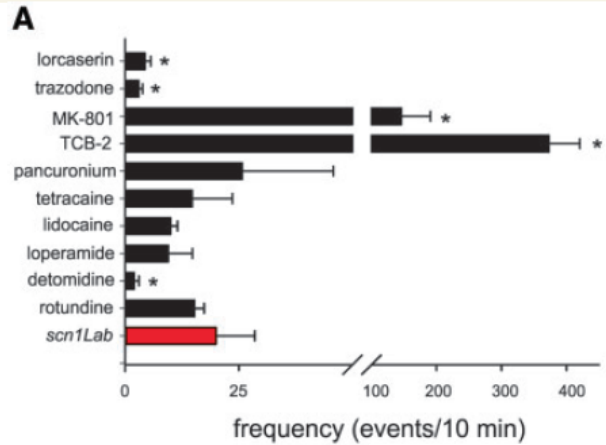
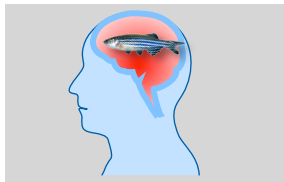


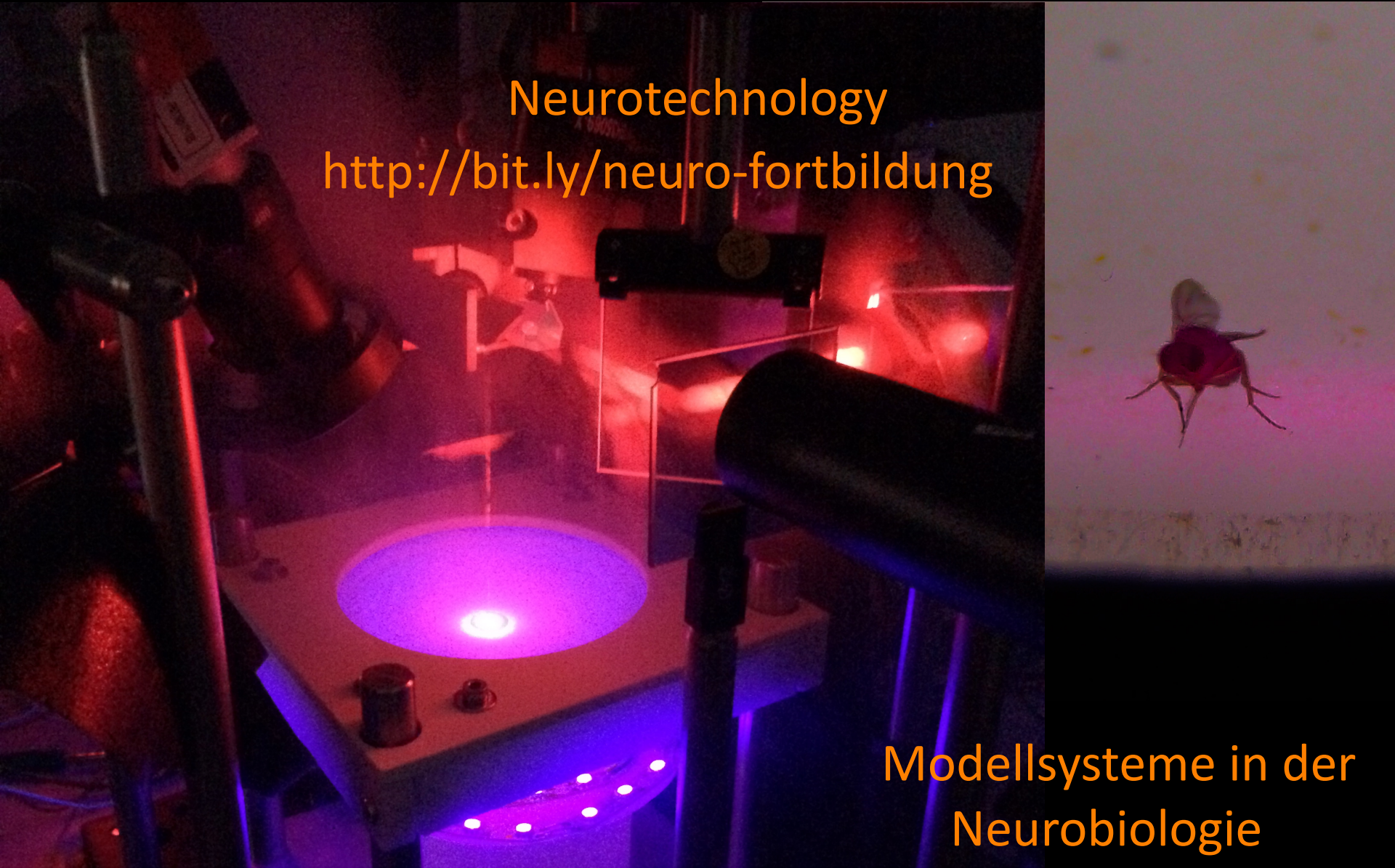
Table 1 Dravet Syndrome patients treated with Belviiq® (lorcaserin) show reduced seizure frequency

Patient	1	2	3	4	5
Age (years)	10	18	10	7	14
Weight (kg)	28	46	23	24	35
Dose (mg/kg/day)	0.25	0.27	0.19	0.32	0.31
Prior treatments	CLZ CZP KD LMT LVT PRM OXC RUF TPX VPA	CBZ CBD CLZ CLB CZP FBM LMT LVT PRM PHB TPM VPA CC KD VNS	ESM FBM LMT LVT MSM VPA VMP ZNM KD	CZP ESM LVT LZP STP TPM ZNM KD	CBZ FBM GBP LCM LMT LVT OXC PHB PRED RUF STP VNS VPM ZNM KD
Concurrent AEDs	CLB STP VPA	CZP STP ZNM	KD TPM VPA	BRO CBD CLB VPA	CLB TPX VPA
Prior seizure frequency	FS: 50/day	MS: numerous daily	MS: daily	AS: 12/h	MS: constant throughout the day
	GTC clusters: 1/ month	FS + GTC: 10/ month (requires rescue medications)	GTC seizures: 100/ month (clusters 7-10)	FS: 3-5 / week	GTC seizures: 1-2/ week
Seizure frequency after treatment: first 3 months	Seizure free initial 3 weeks, cluster of seizures then again seizure free for 2 weeks	Seizure free for 2 weeks	GTC seizures: 46/ month (GTC clusters of 1-3 seizures)	NCS: 1/ month 1-2 seizure free days/ week	MS: initially reduced in the morning then increases to constant throughout the late afternoon



Diskussion

- Vielen Dank für Ihre Aufmerksamkeit



Neurotechnology
<http://bit.ly/neuro-fortbildung>

Modellsysteme in der
Neurobiologie



Andrew Straw

Biologie I & Bernstein Center Freiburg

www.strawlab.org

NWG-Lehrerfortbildung

4 Oct 2017

“Progress in science depends on new techniques, new discoveries and new ideas, probably in that order.”

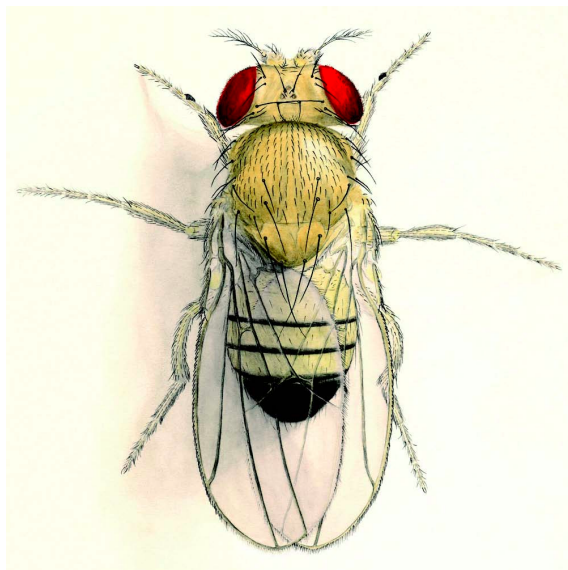
– Sydney Brenner, Winner 2002 Nobel Prize in Physiology or Medicine

The role of technology in basic neuroscience research.

Translation from research to application.

Applications:

- **Human health**
- **Automation and robotics**



“The key fourth awardee here is the little fly.” -Jeffrey C. Hall, Winner 2017 Nobel Prize in Physiology or Medicine

In model organisms:

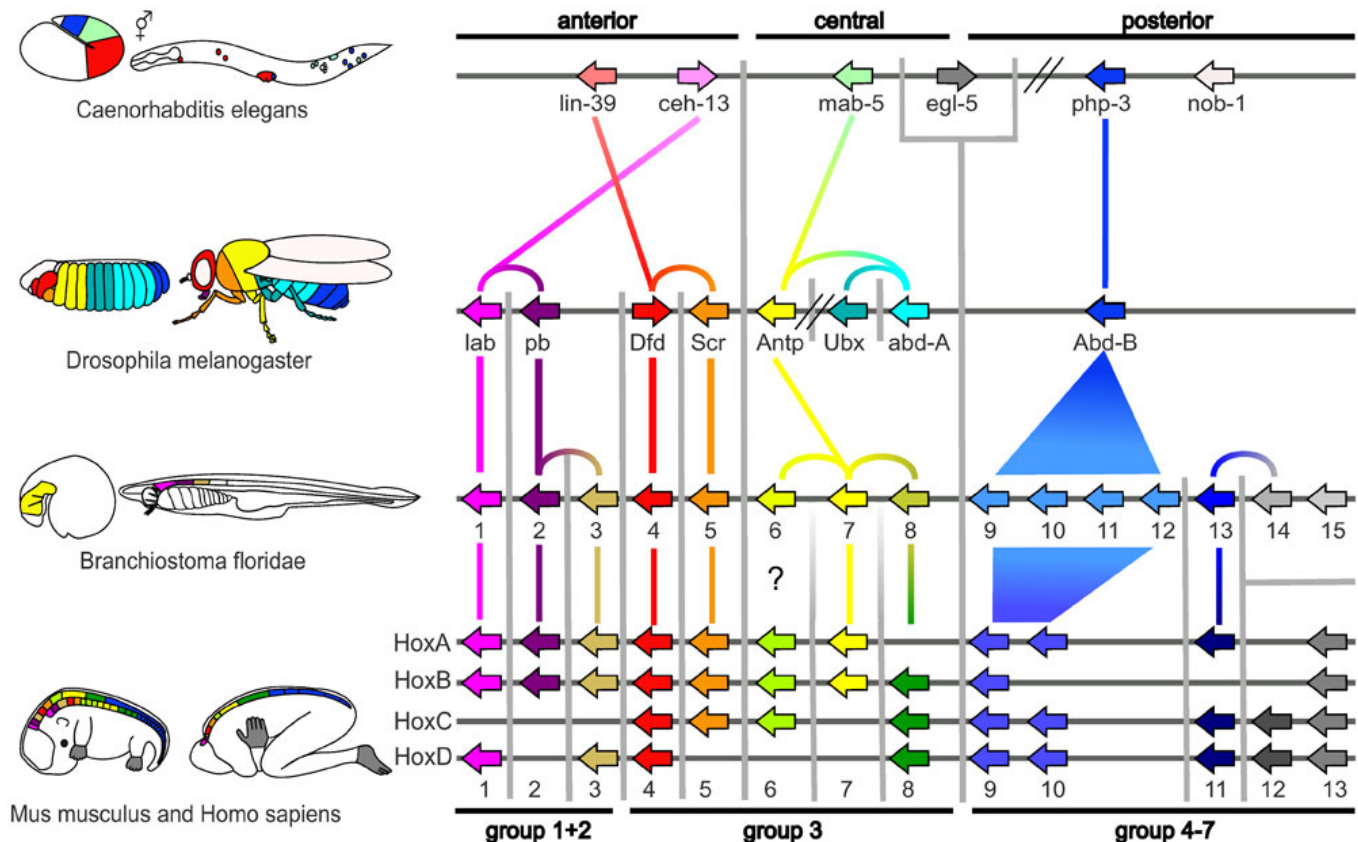
Technology development

Discovery of basic principles

“Bang for buck”

Biggest surprise of biology in last 100 years:

Deep shared ancestry of genes, development and brains



Example of neurotechnology

Advanced genetic tools – precise targeting & manipulation

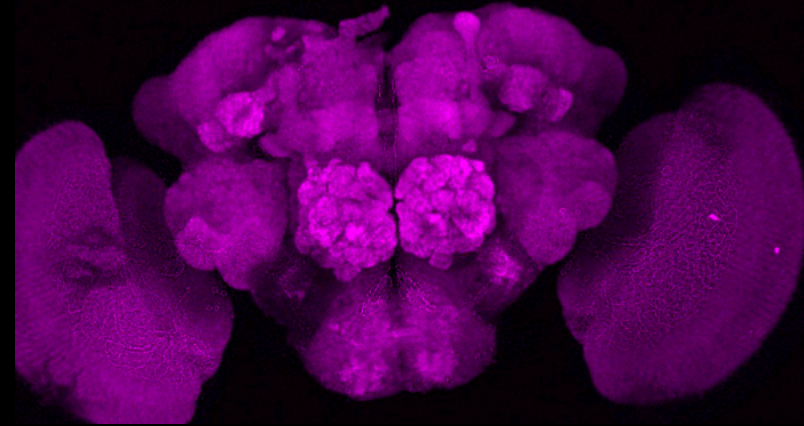
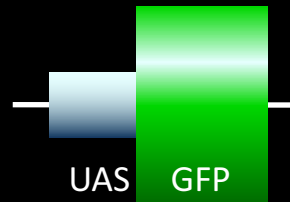
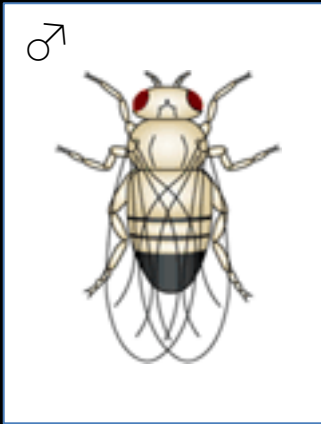
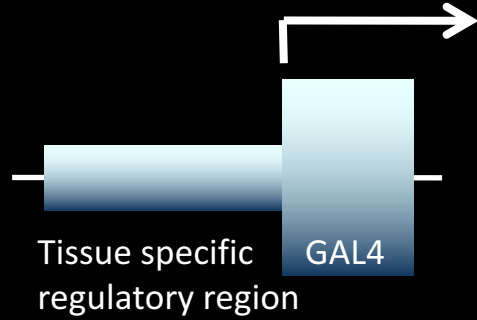
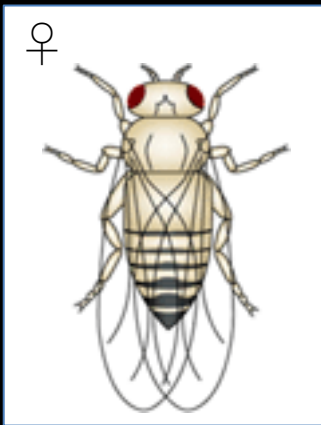
Novel tool ‘FlyMAD’

Persistent arousal from brief neural activation

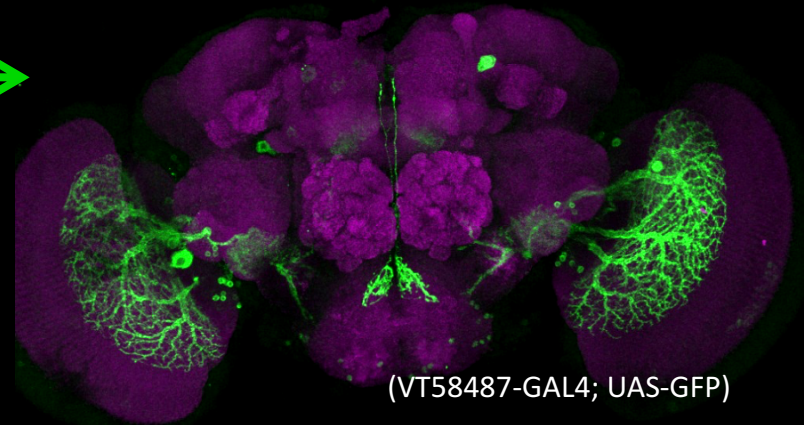
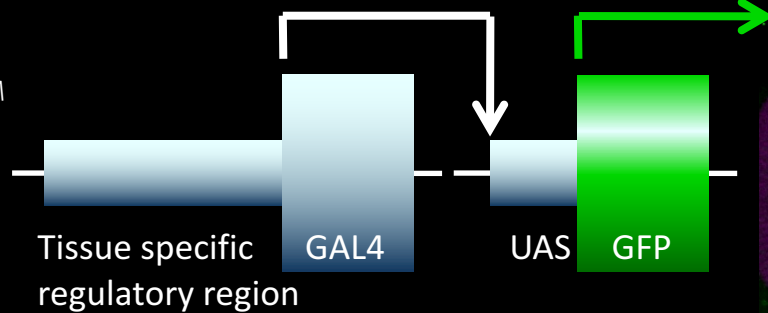
Bath DE*, Stowers JR*, Hörmann D, Poehlmann A, Dickson BJ[†], Straw AD[†]
(2014) FlyMAD: Rapid thermogenetic control of neuronal activity in freely-walking *Drosophila*. *Nature Methods*

(* equal contribution, (†) co-corresponding author

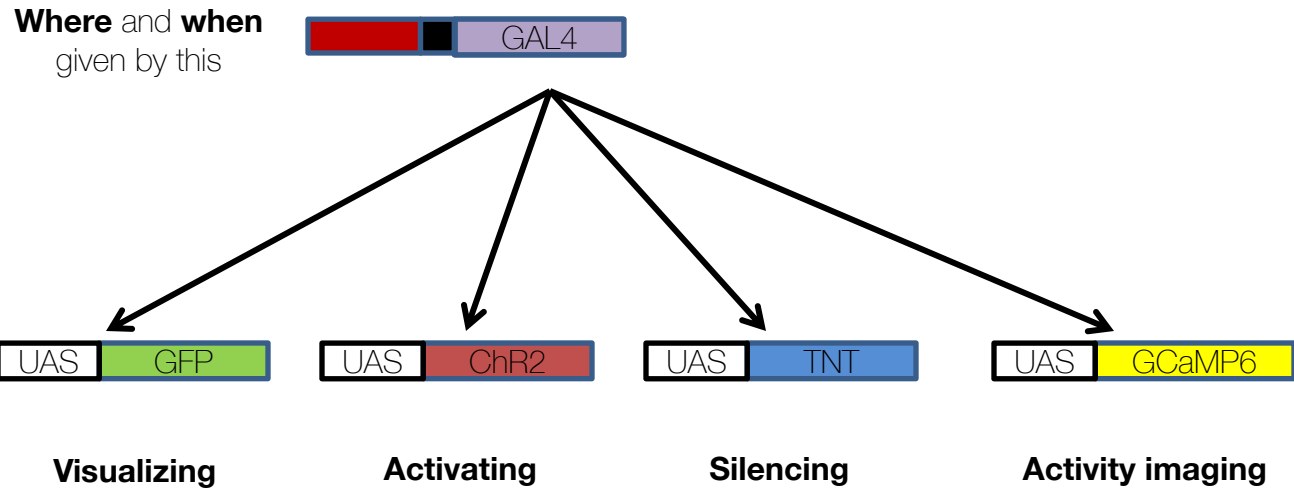
Genetics – GAL4/UAS



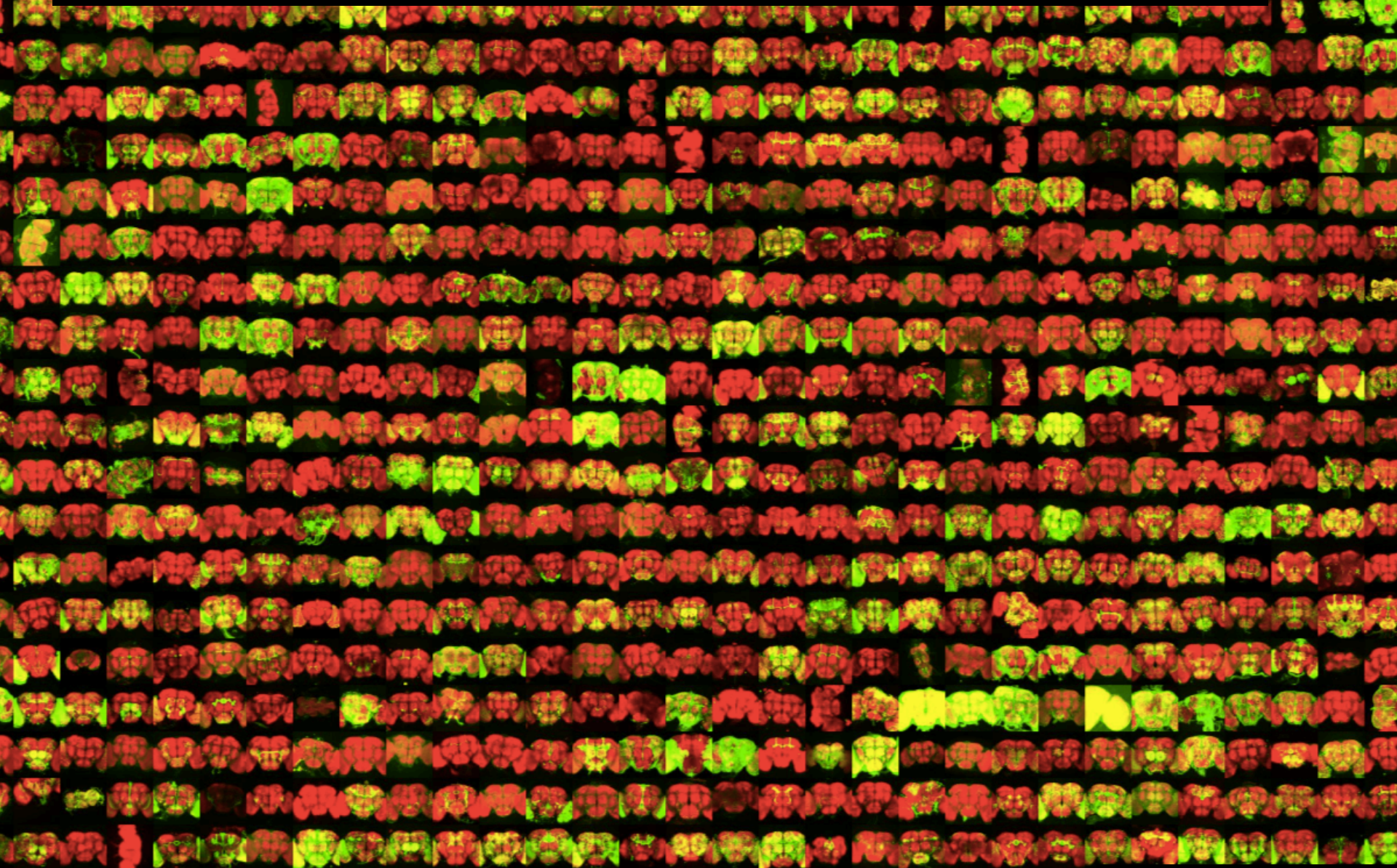
offspring ♀ x ♂



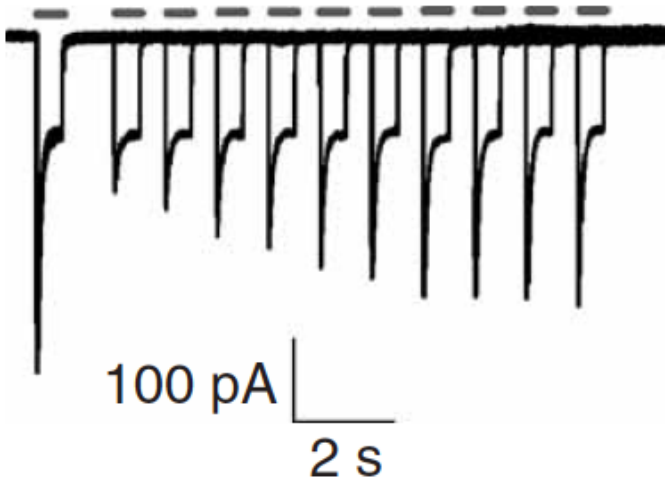
the modular power of Gal4-UAS



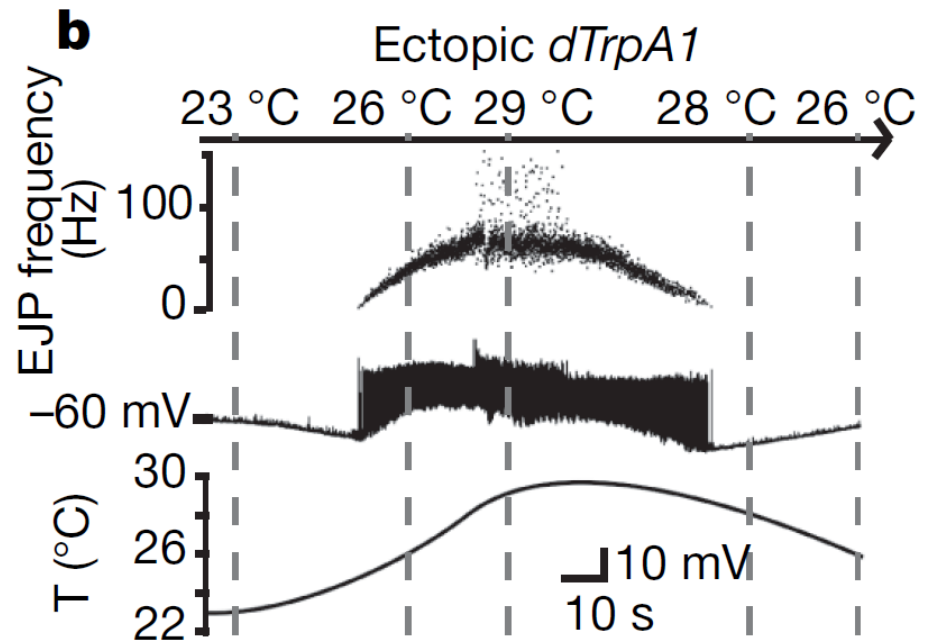
GAL4 driver lines (“Treiberlinie” oder “Treiberstämme”)



Optogenetics & Thermogenetics

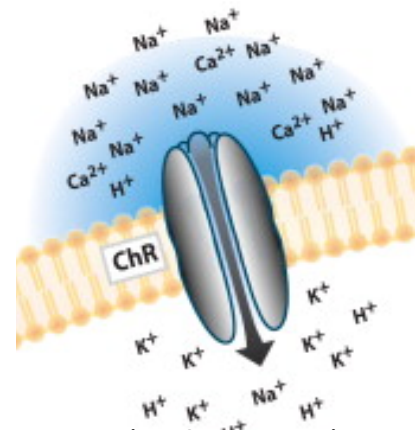


Boyden ... Deisseroth *Nature Neuroscience* 2005



Hamada...Garrity
Nature 2008

Where do our optogenetic tools come from?



From Yizhar & Deisseroth, 2011

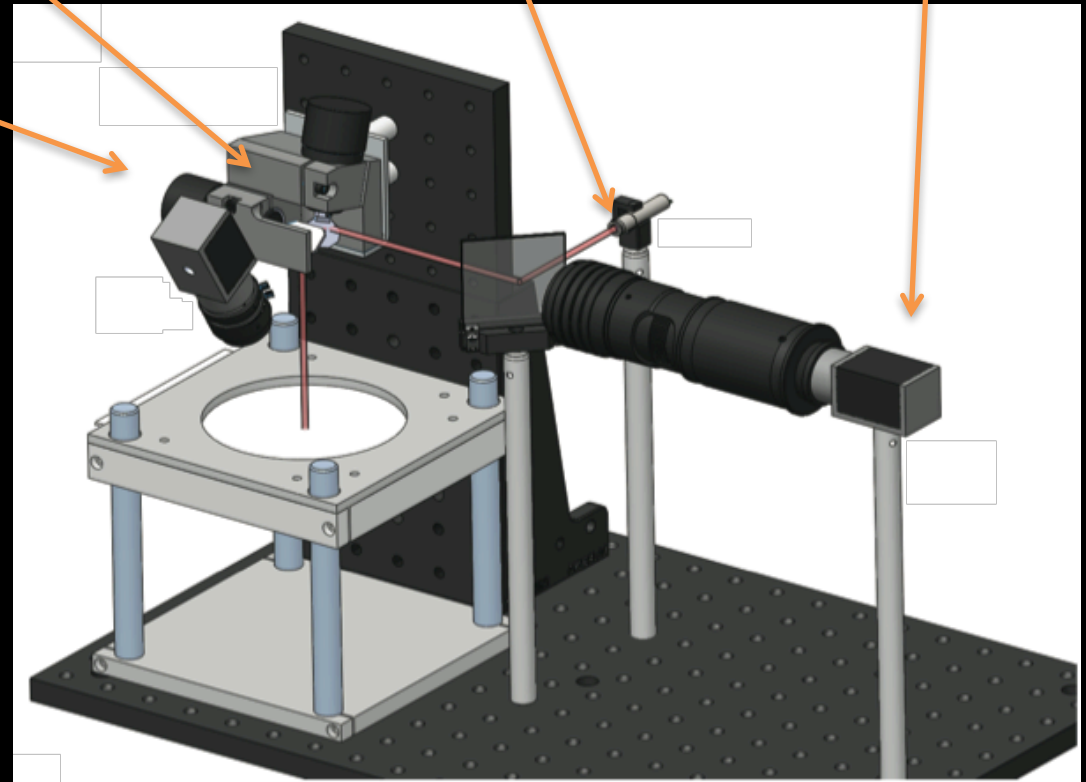
FlyMAD – the Fly Mind Alteration Device

mirror-steerable
optical path

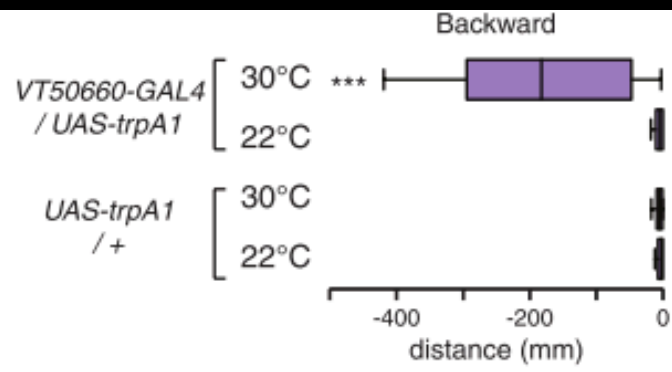
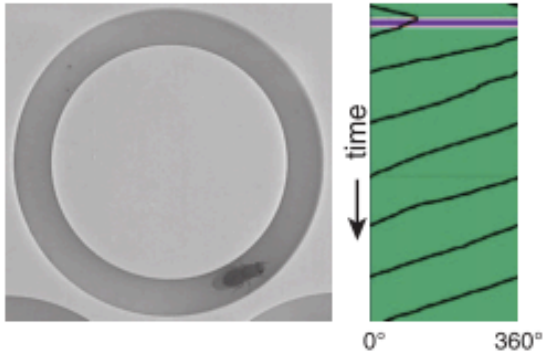
laser (808nm
350mW)

through-the-
mirror camera

wide-field
tracking
camera



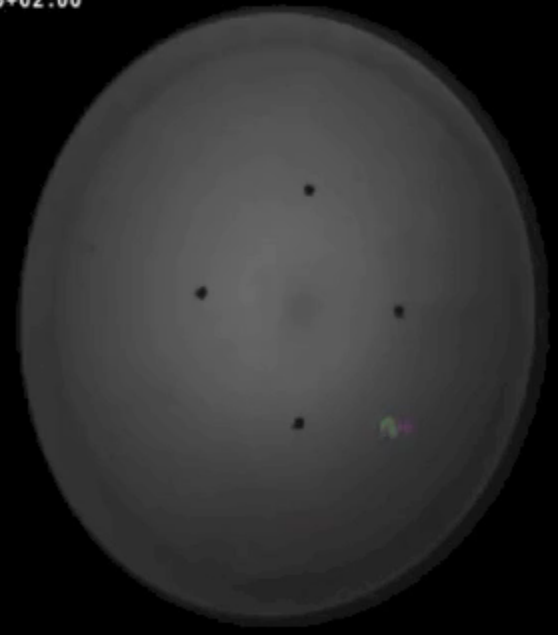
walking fly



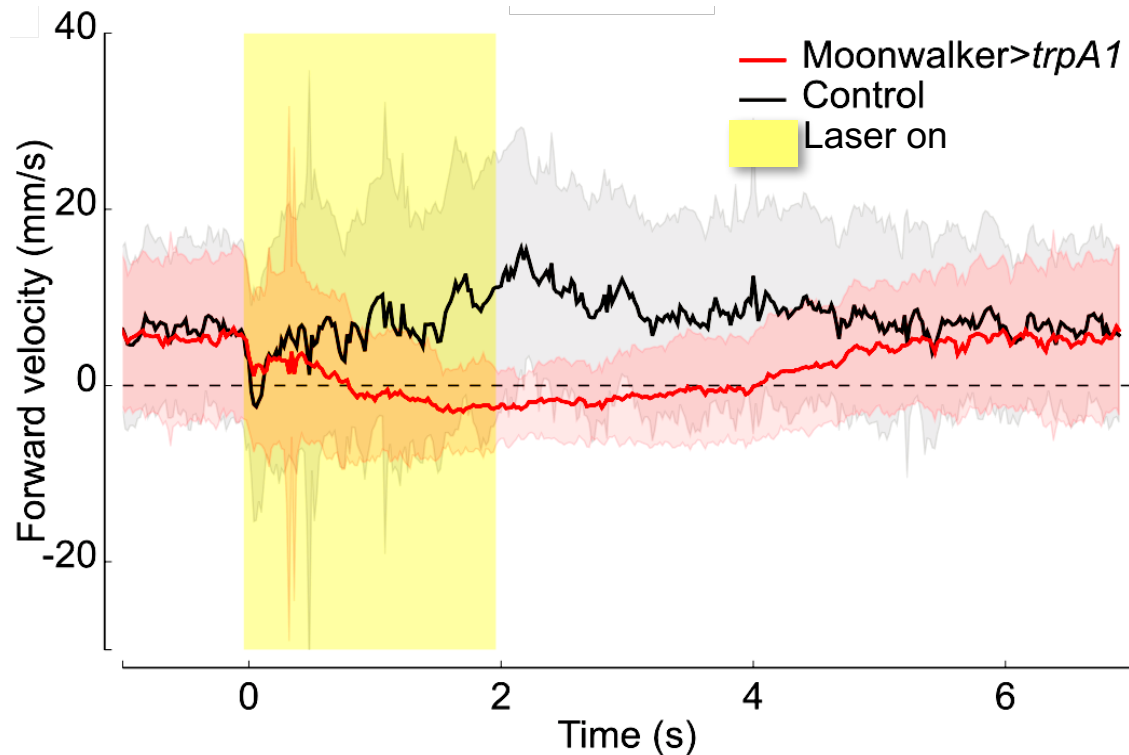
Bidaye *et al.*, *Science* 2014

Moonwalker>TrpA1

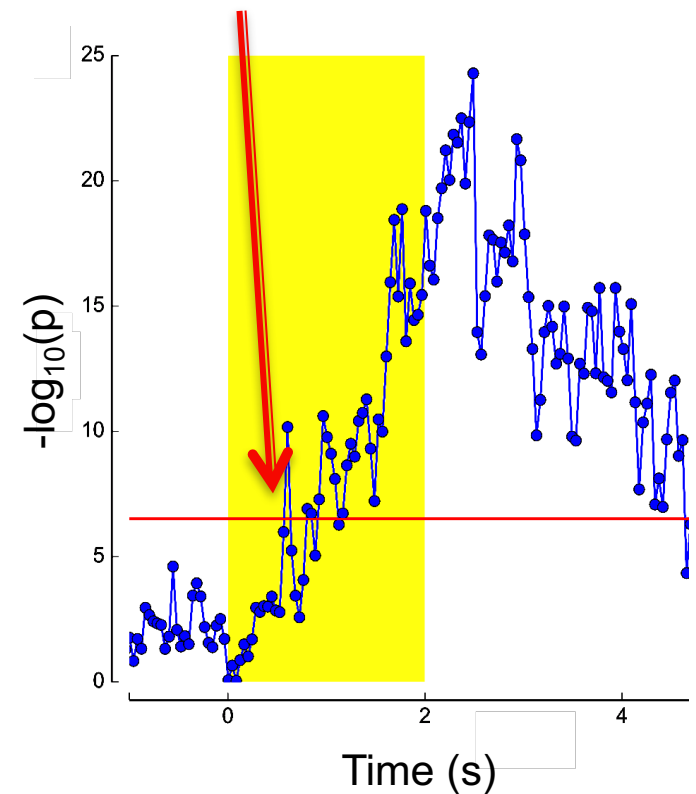
2013-09-04 12:58:53.126726+02:00



'Moonwalking' with activator



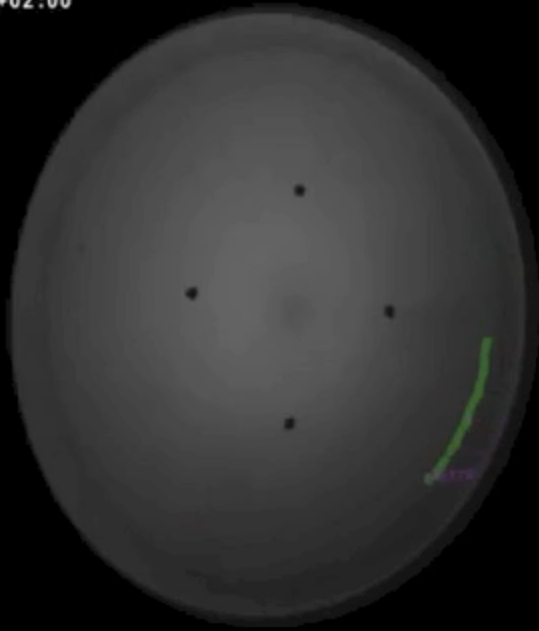
0.7 seconds



VT50660-GAL4/UAS-trpA1 (red, $n=13$ flies, 10 trials per fly) and pooled control ($+/UAS-trpA1$ and *VT50660-GAL4/+*; black, $n=8$ flies, 10 trials per fly)

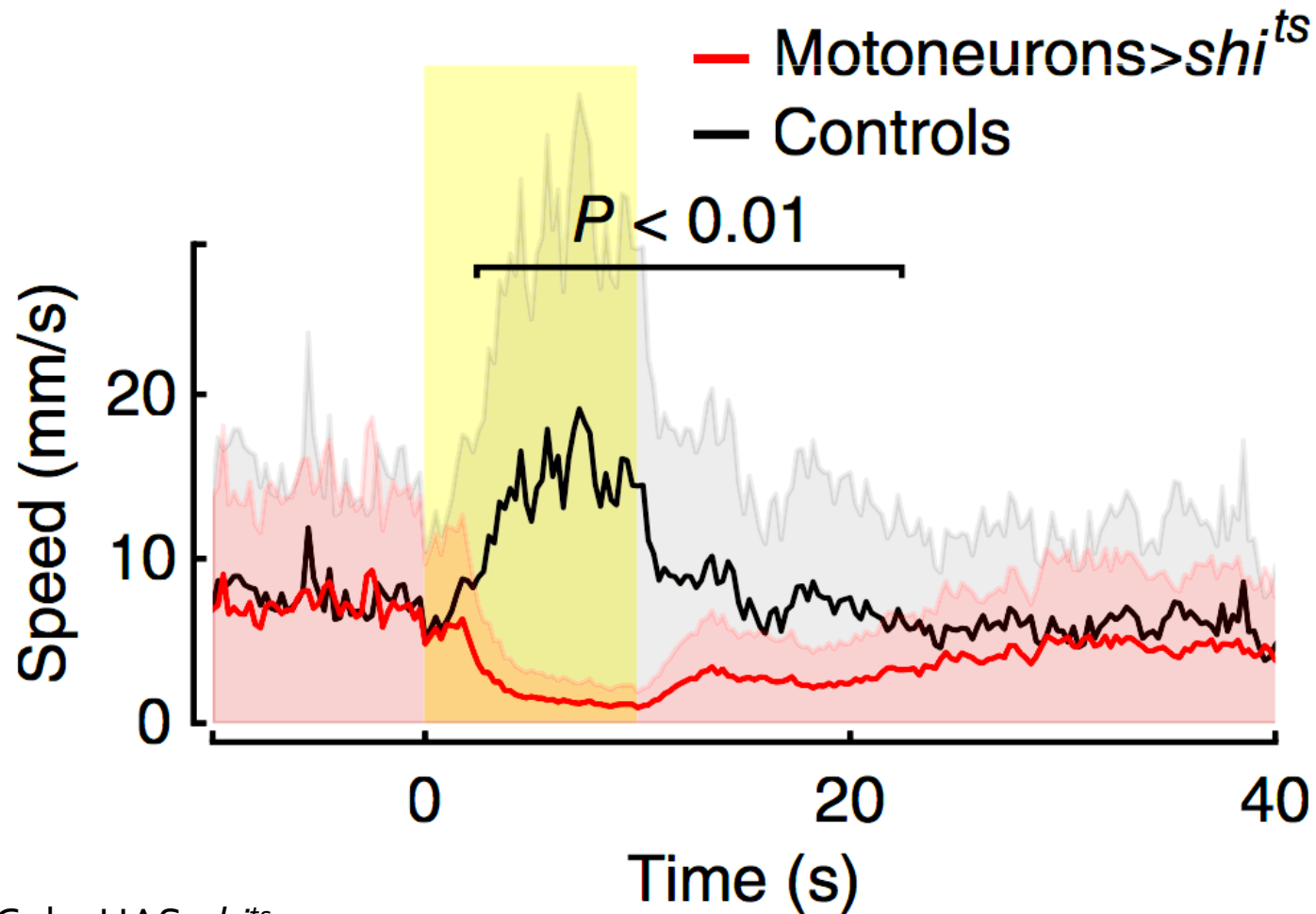
Motorneurons>shibire^{ts}

2013-09-11 17:23:08.056792+02:00

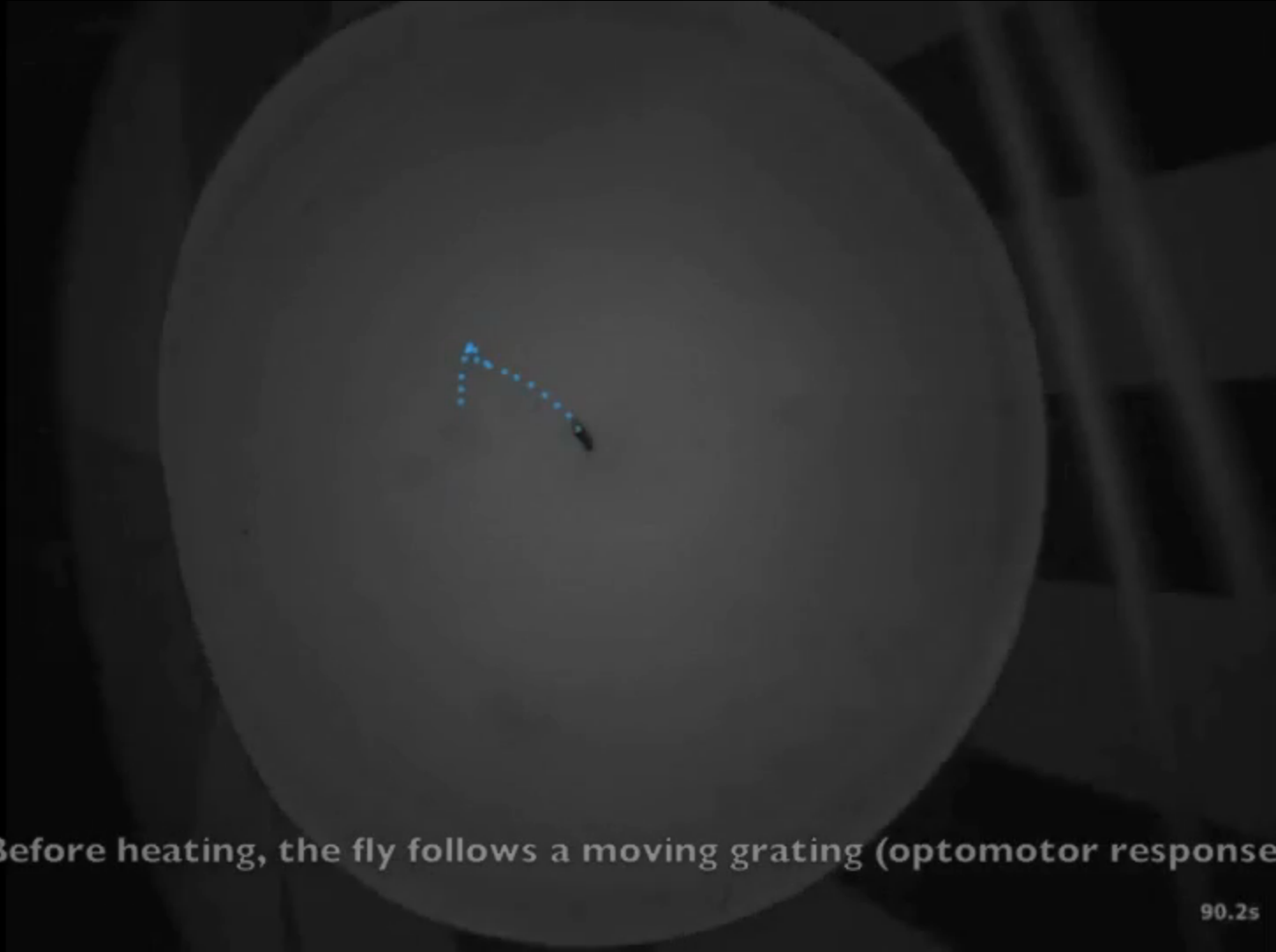


2x playback speed

Silencing motor neurons with ShibireTS stops flies



R1-6 photoreceptors>shibire^{ts}

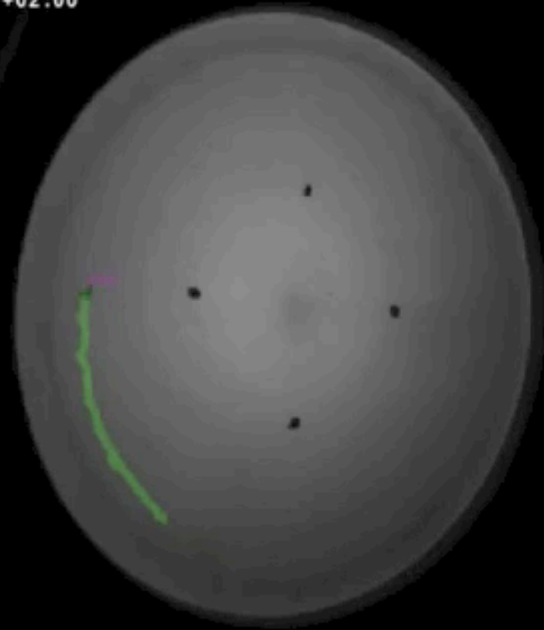


Before heating, the fly follows a moving grating (optomotor response).

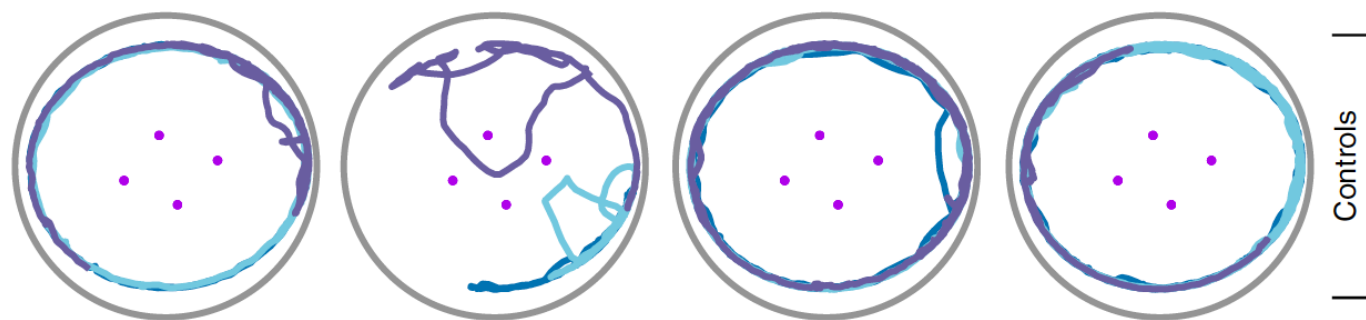
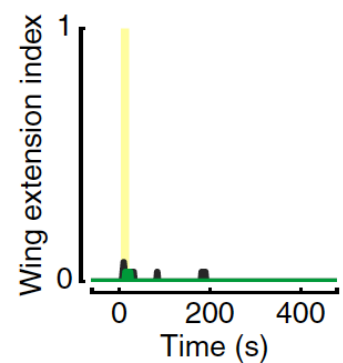
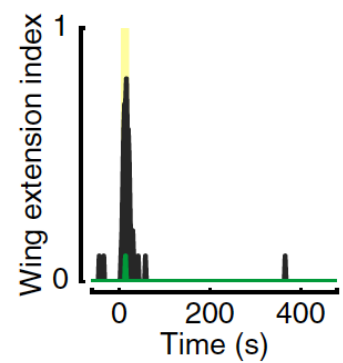
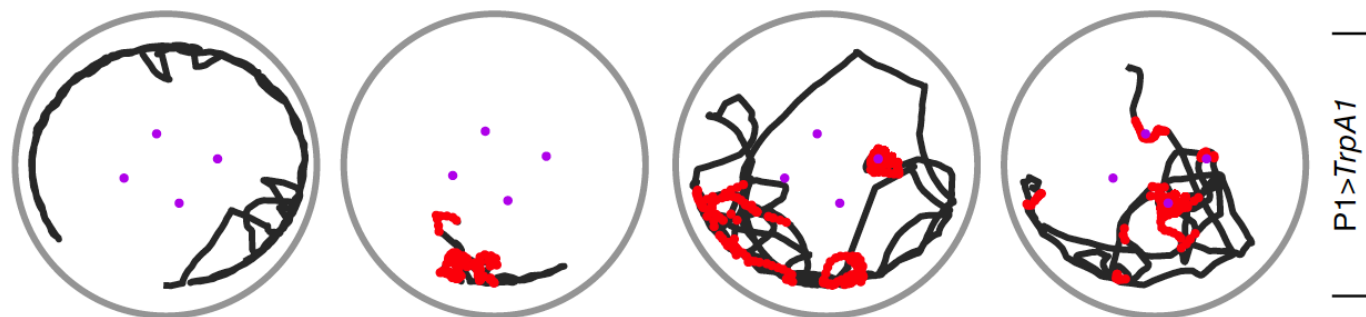
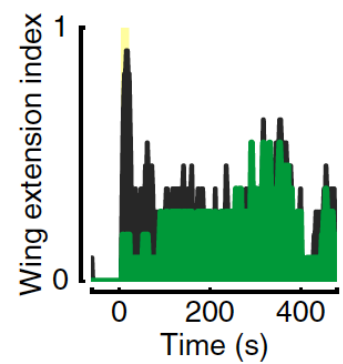
90.2s

Persistent courtship after brief activation of P₁ neuron

2013-08-23 12:59:48.648347+02:00



0000



Before During Early Late

— UAS control — P1 GAL4 control — pIP10 GAL4 control — Fly position — Wing extended

Example of neurotechnology

Advanced genetic tools – precise targeting & manipulation

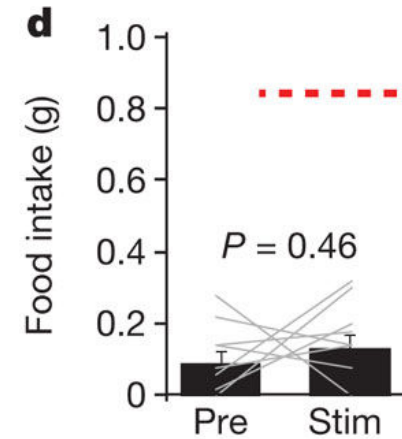
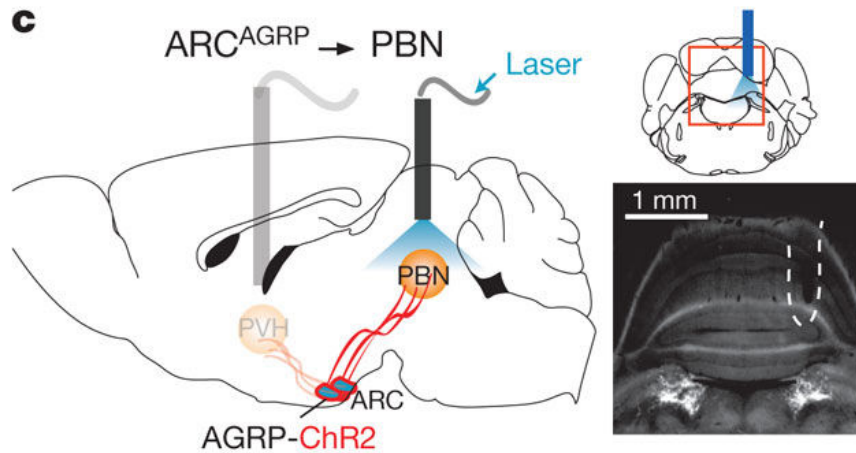
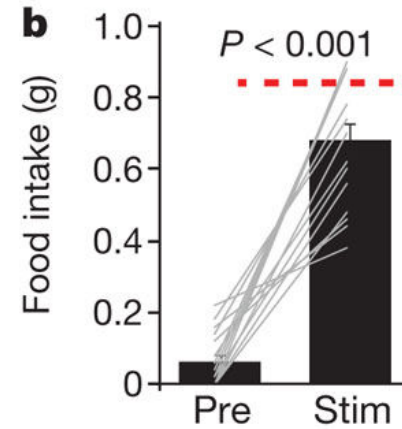
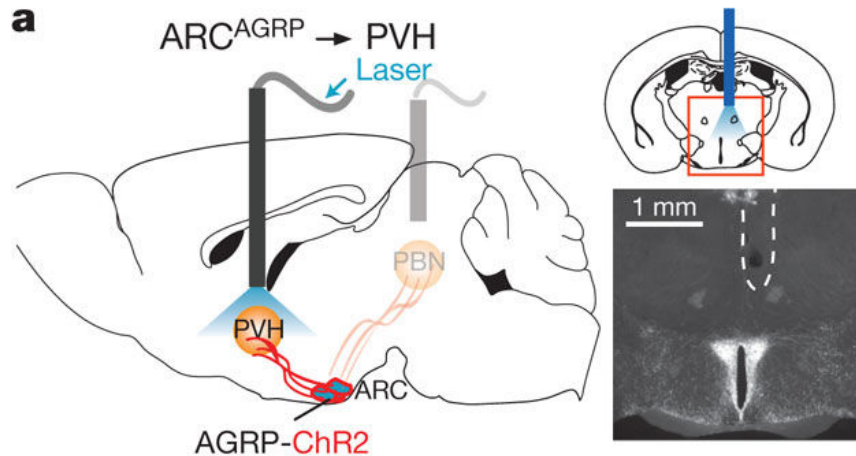
Novel tool ‘FlyMAD’

Persistent arousal from brief neural activation

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(2014) FlyMAD: Rapid thermogenetic control of neuronal activity in freely-walking *Drosophila*. *Nature Methods*

(* equal contribution, (†) co-corresponding author

Conceptually similar neurotechnology in mouse



Adeno-associated virus (AAV)

Can infect neurons

Low pathogenicity

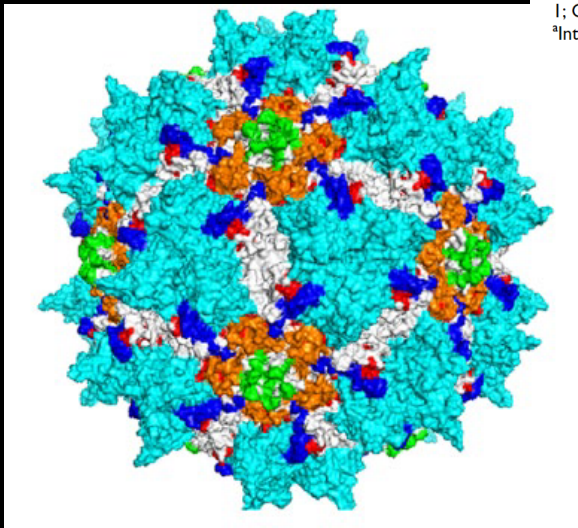
Can deliver 4.8 kB DNA without viral DNA

Table 1. Summary of AAV Clinical Trials in the CNS.

Disease	Clinical Trial	Serotype	Promoter	Transgene Product	ClinicalTrials.gov identifier	Refs
Alzheimer's	Phase 2	AAV2	CAG	Nerve growth factor (NGF)	NCT00876863	Mandel and others, 2010
Canavan	Phase 1	AAV2	NSE	Aspartoacylase (ASPA)	NA	McPhee and others, 2006
Late infantile neuronal ceroidlipofuscinosis	Phase 1	AAV2	CAG	Human CLN2	NCT00151216	Worgall and others, 2008
Late infantile neuronal ceroidlipofuscinosis	Phase 1/2 recruiting	AAVrh.10	CAG	Human CLN2	NCT01414985	
Parkinson's	Phase 1/2	AAV2	CAG	Neurturin (NTN)	NCT00400634 NCT00985517	Bartus and others, 2013
Parkinson's	Phase 1 recruiting	AAV2	NA	Glial cell-derived neurotrophic factor (GDNF)	NCT01621581	
Parkinson's	Phase 2	AAV2	CAG	Glutamate decarboxylase 1 (GAD)	NCT00643890	LeWitt and others, 2011
Sanfilippo syndrome (MPSIIIB)	Phase 1/2 recruiting	AAV5	NA	Human α -N-acetylglucosaminidase	ISRCTN ^a 19853672	Ellinwood and others, 2011

Abbreviations: AAV = adeno-associated virus; CAG = chicken β -actin promoter with CMV enhancer; CLN2 = also known as tripeptidyl peptidase I; CMV = cytomegalovirus; NA = not available; NSE = neuron-specific enolase.

^aInternational Standard Randomized Controlled Trial Number.



Key points - Neurotechnology

**Advanced genetic tools – precise targeting & manipulation
GAL4+UAS (flies) and AAV+optogenetics (mammals)**

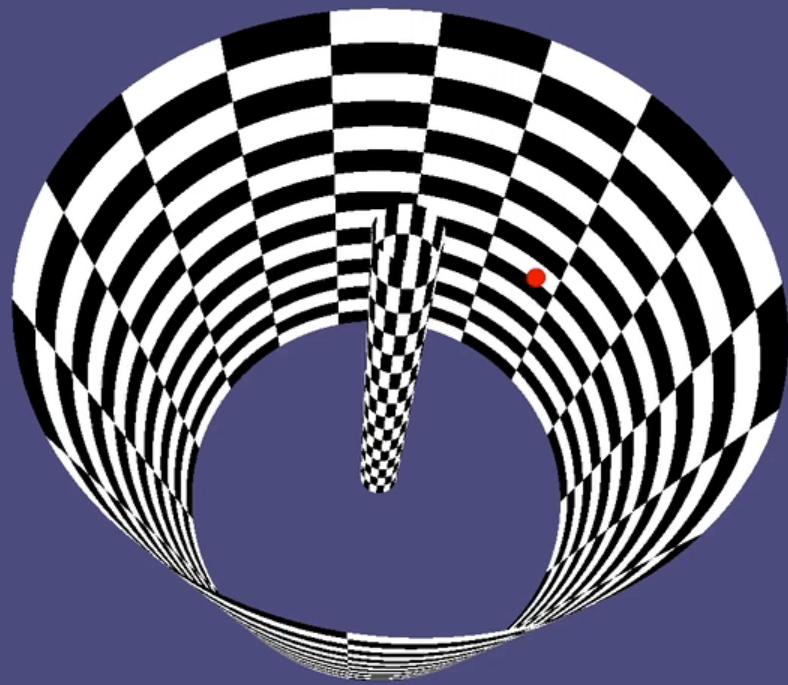
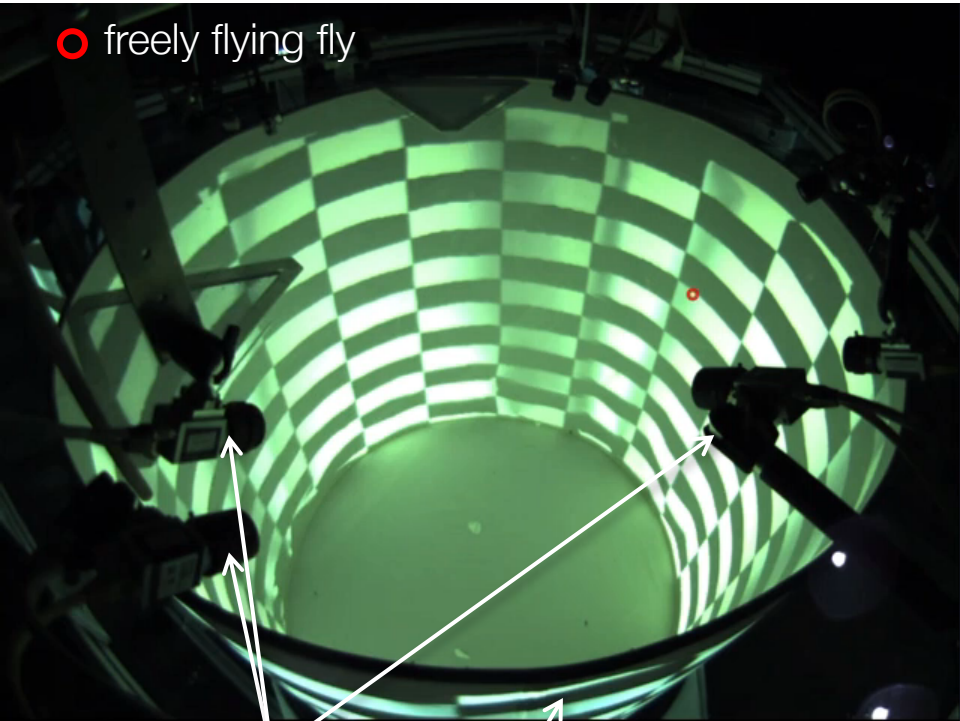
**Novel technical and computer tools enable new discoveries
(automated behavior analysis)**

**Persistent brain states can be triggered by brief stimulation
(courtship in flies, eating in mice)**

A central aim in neuroscience is to understand the mechanistic basis of behavior.

Our lab develops and uses virtual reality to study behavior in quantitative detail.

○ freely flying fly

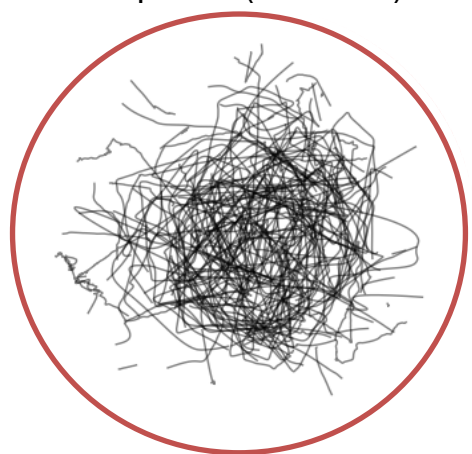
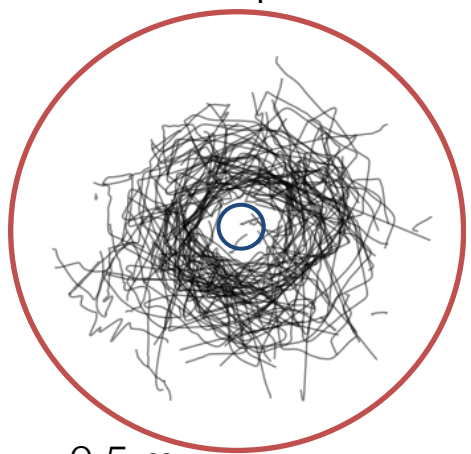


high-speed cameras

computer projected image

virtual post

no post (control)



0.5 m



It has been difficult to directly manipulate interactions between organisms.

Free-moving VR allows new ways to study social behavior



virtual fish



Renaud Bastien
Iain Couzin (Uni Konstanz/MPI)

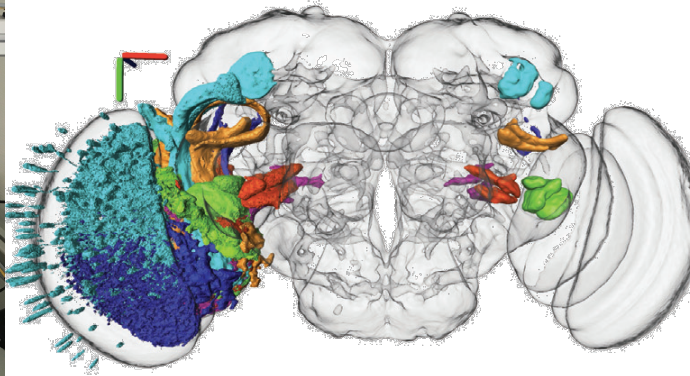
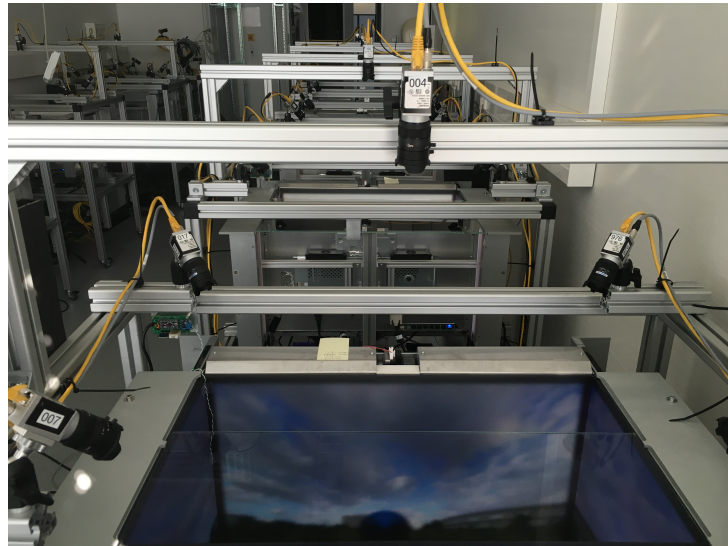
Stowers*, Hofbauer* ... Tessmar-Raible**, Straw** *Nature Methods* (in press)

*equal contribution **corresponding author

Model systems - very important for basic and applied neuroscience

<http://bit.ly/neuro-fortbildung>

Straw Lab: behavior, genetics, anatomy, physiology



MC61, LC10

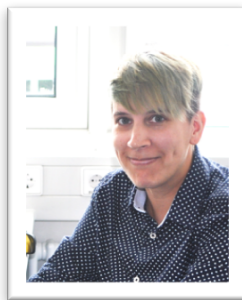
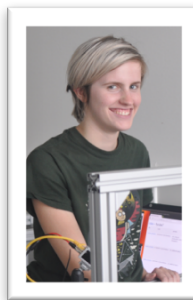
LC06, LC16

LPC1, LPLC3, LC22/LPLC4

LC12, LC17, LC18

LPLC1, LPLC2, LC04

MC62, MC63



Andreas Poehlmann

Etienne Campione

Sayan Soselisa

Katja Hellekes

Abina Boesjes

Andreas Balog

Adrian Hiss

