New approaches for neurochemical monitoring

- Technical Journal Club -

Silvia Sorce

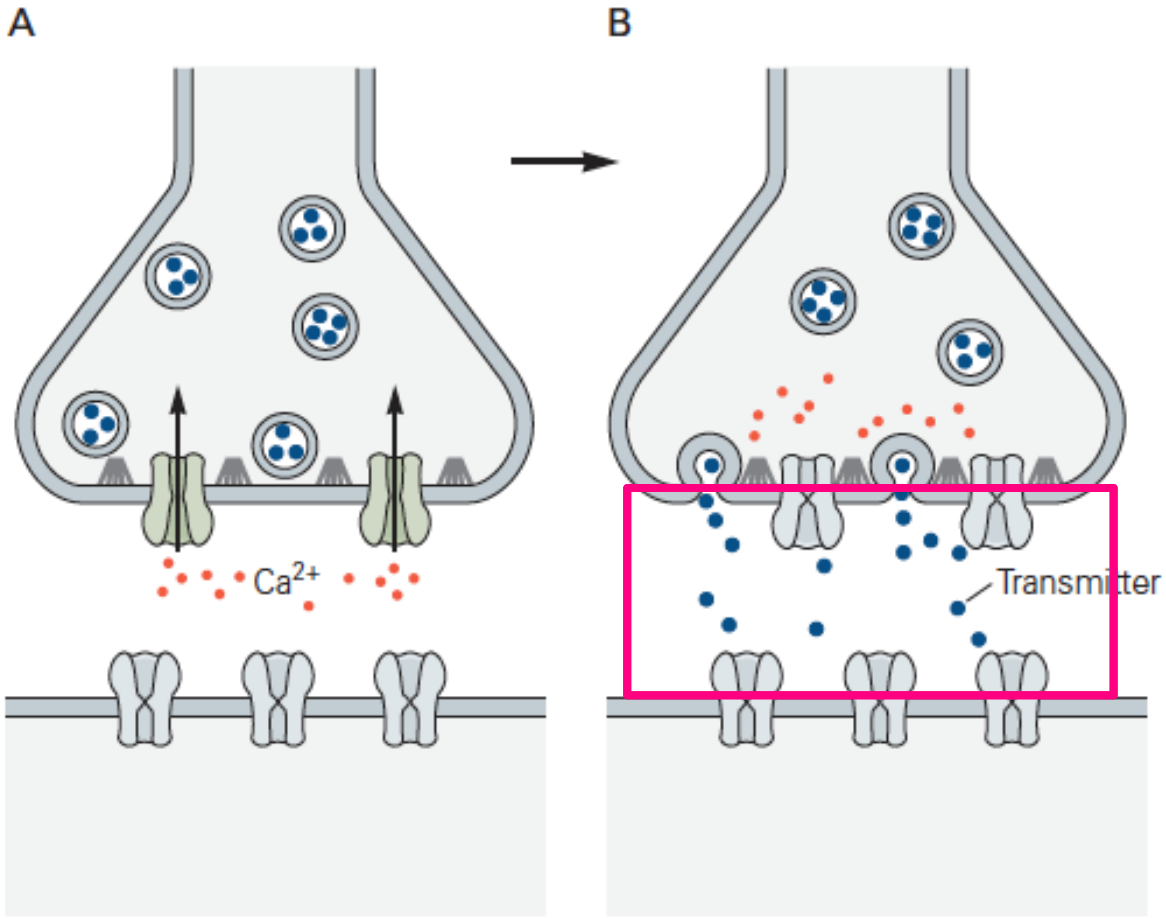
9th December 2014

Outline

- Introduction
- Fast scan cyclic voltammetry
- Cell-based neurotransmitter fluorescent engineered reporters (CNiFERs)

Background: synaptic transmission

→ a neurotransmitter can be defined as a substance that is released by a neuron and that affects a specific target in a specific manner



How much/When is released? → chronic, real-time neurochemical monitoring

Kandel *et al.*, Principles of Neural Science, 5th edition, 2013

Background: neurotransmitters

Table 13–1 Small-Molecule Transmitter Substances and Their Precursors

Transmitter	Precursor
Acetylcholine	Choline
Biogenic amines	
Dopamine	Tyrosine
Norepinephrine	Tyrosine
Epinephrine	Tyrosine
Serotonin	Tryptophan
Histamine	Histidine
Melatonin	Serotonin
Amino acids	
Aspartate	Oxaloacetate
γ-Aminobutyric acid	Glutamine
Glutamate	Glutamine
Glycine	Serine
ATP	ADP
Adenosine	ATP
Arachidonic acid	Phospholipids
Carbon monoxide	Heme
Nitric oxide	Arginine

Acetylcholine is released at all vertebrate neuromuscular junctions by spinal motor neurons

Cholinergic neurons → widespread projections to the cerebral cortex; modulates arousal, sleep, wakefulness, and other critical aspects of human consciousness

Kandel *et al.,* Principles of Neural Science, 5th edition, 2013

Background: neurotransmitters

Table 13–1 Small-Molecule Transmitter Substances and Their Precursors

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Glutamate	Glutamine
Glycine	Serine
ATP	ADP
Adenosine	ATP
Arachidonic acid	Phospholipids
Carbon monoxide	Heme
Nitric oxide	Arginine

Dopamine is released from the dopaminergic neurons in the ventral midbrain towards different regions, in particular striatum/nucleus accumbens → Regulate motor, emotional, attentional, cognitive, reward-related behaviors

Norepinephrine is released from noradrenergic neurons in the locus coeruleus in CNS. Most responsible for vigilant concentration, reaction to stimuli. Released also from the sympathetic neurons to control fight-or-flight response

Kandel *et al.*, Principles of Neural Science, 5th edition, 2013

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Fast scan cyclic voltammetry

Small electrode inserted in brain region of interest

Carbon-fiber microelectrodes (diameter of 5 µm) are the most common sensors used in this application → encased in a pulled glass capillary with 25-100 µm of the fiber protruding from the glass

Dopamine, norepinephrine, epinephrine, serotonin, and histamine are easily oxidized and thus can be detected with voltammetric experiments

High temporal resolution detection (subseconds)

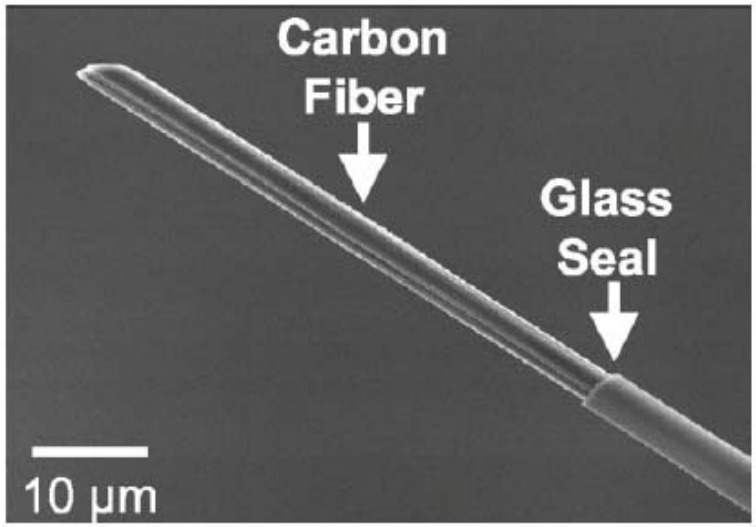


Fig. 2. Scanning electron microscopic image of a cylindrical carbon-fiber microelectrode under ×1200 magnification.

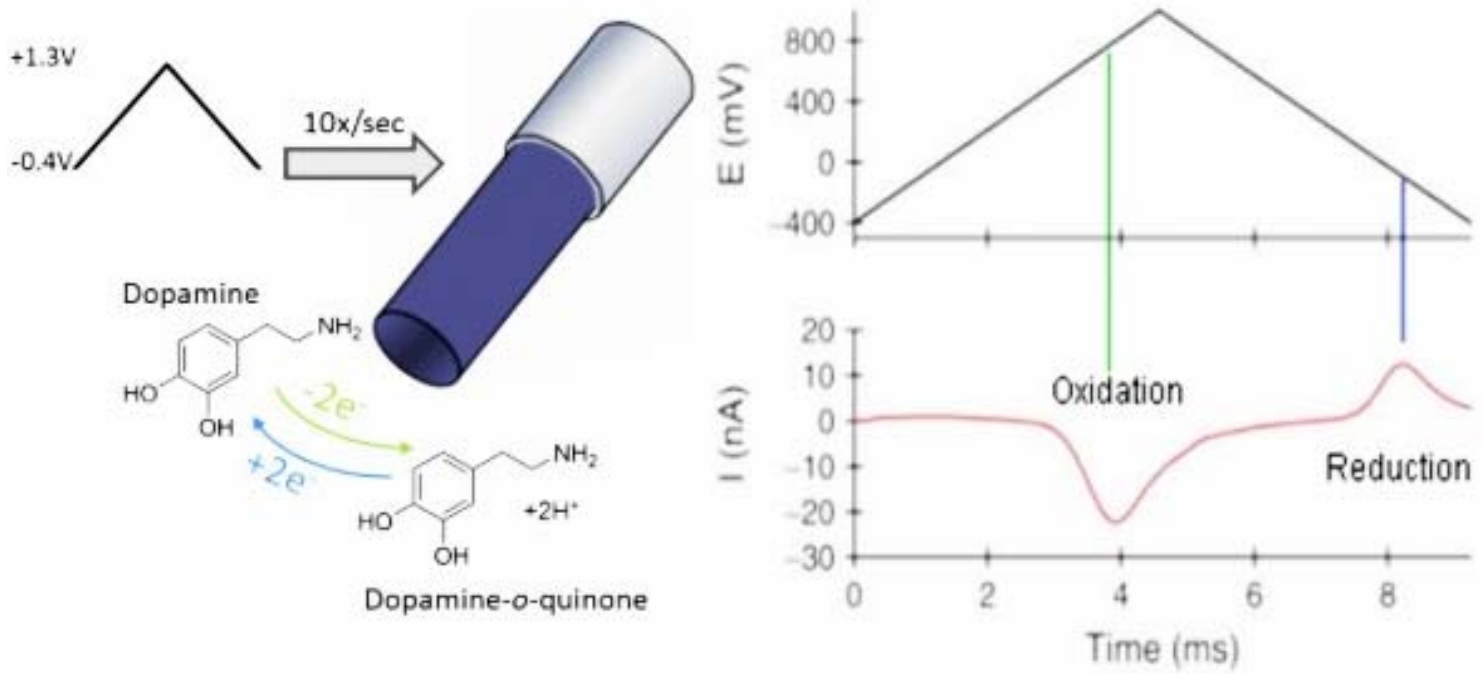
The diameter of the fiber is ~5 µm. The length of the exposed carbon fiber extending from the glass seal is the electroactive area of the electrode.

Robinson et al., Clin Chem, 2003

Fast scan cyclic voltammetry

Voltammetry: current (I) is measured as a function of voltage (E).

1. a potential is applied to the electrode



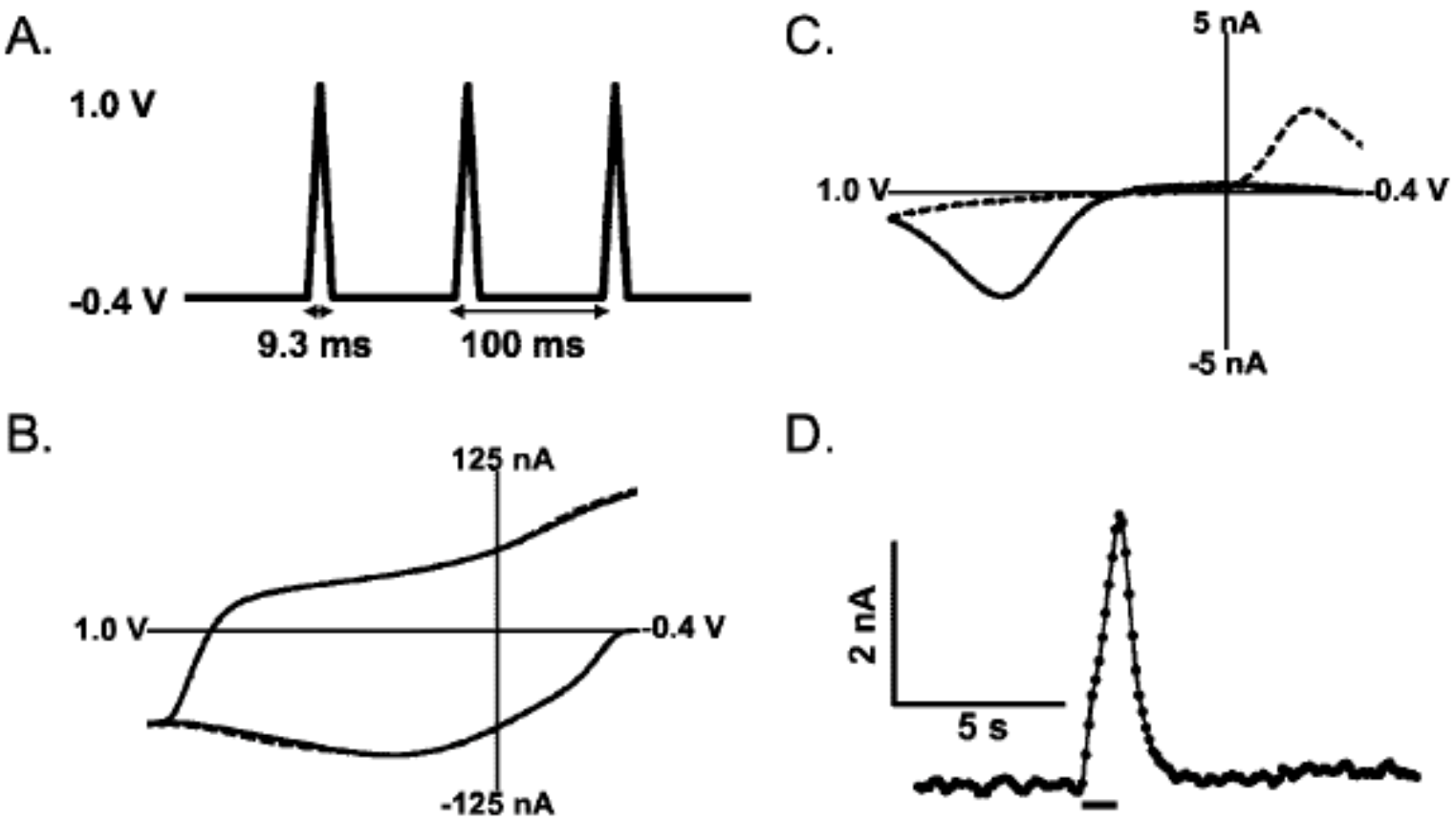
2. during the positive sweep dopamine is oxidized to form dopamine-o-quinone (peak reaction at approximately +0.64 V).

3. Dopamine-o-quinone is then reduced back to dopamine in the negative sweep (peak reaction at approximately -0.2 V).

The ensuing flux of electrons is measured as current and is directly proportional to the number of molecules that undergo the electrolysis.

http://wassumlab.psych.ucla.edu/

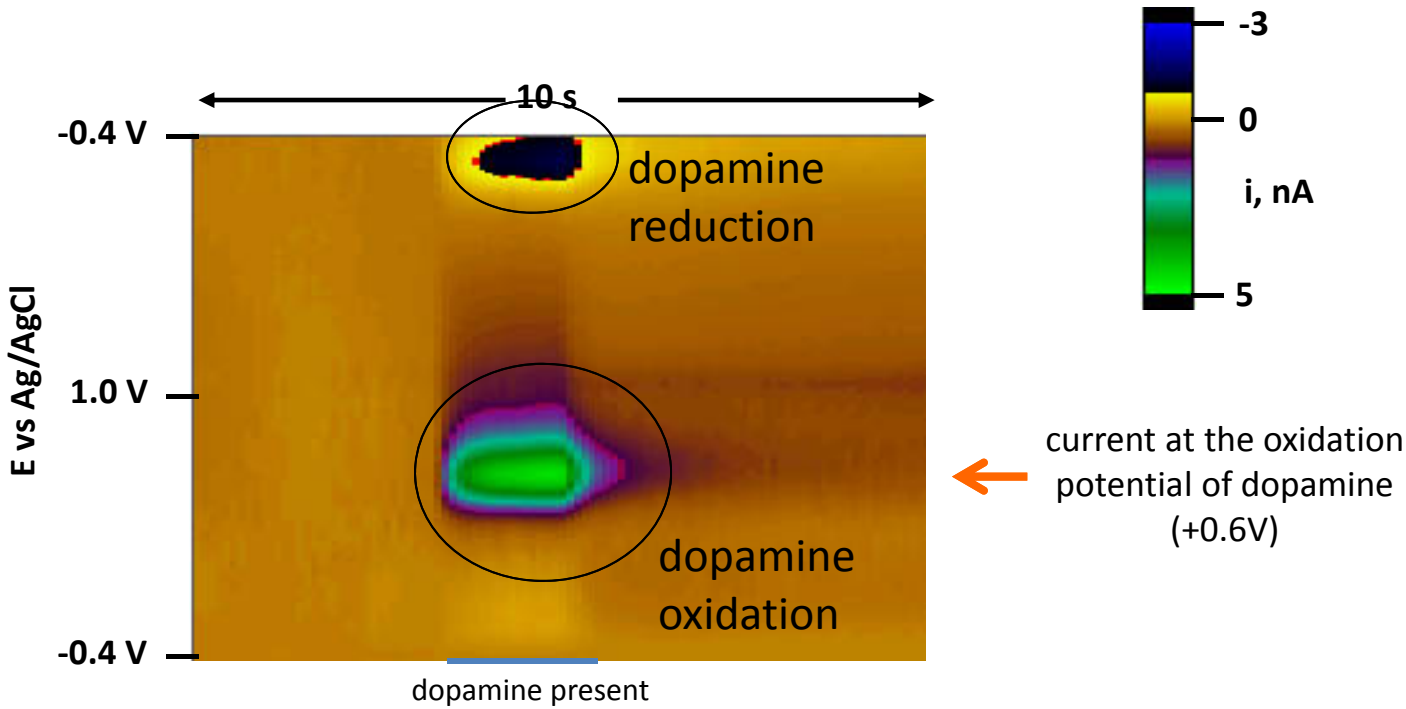
Fast scan cyclic voltammetry: cyclic voltammogram



A. The potential applied to the electrode is ramped from 0.4 V to 1.0 V and back
B. Produced large background current, plotted vs applied potential: the solid line is the background current, the superimposed dashed line is the current observed in the presence of dopamine (3% change)
C. By subtracting out the background, a **cyclic voltammogram** for dopamine is generated
D. Changes in dopamine current are visualized by plotting the current at the peak oxidation potential of dopamine vs time. Each point represents the current from one cyclic voltammogram.

Robinson et al., Clin Chem, 2003

Fast scan cyclic voltammetry: color plot



- Data can also be depicted as a color plot to show many CVs over time.
- Current is in color and shows when dopamine is present.

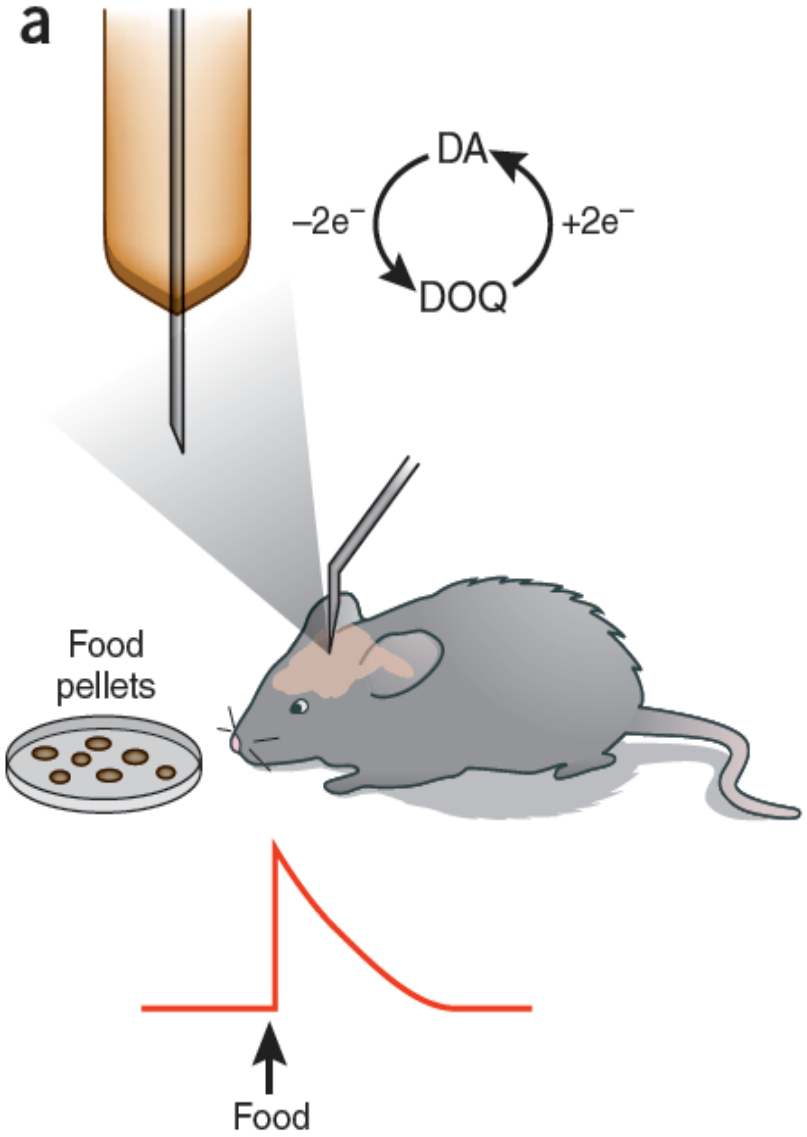
Limitation: requirement for acute implantation of a voltammetric probe into the brain via a microdrive for each experiment → No longitudinal studies possible
the fidelity of chemical recordings can be severely impaired by perturbation of the microenvironment through physical tissue disruption and/or neuroinflammation

Robinson et al., Clin Chem, 2003

Chronic microsensors for longitudinal, subsecond dopamine detection in behaving animals

Jeremy J Clark[1,4], Stefan G Sandberg[1,4], Matthew J Wanat[1], Jerylin O Gan[1,2], Eric A Horne[1], Andrew S Hart[1,2], Christina A Akers[1], Jones G Parker[3], Ingo Willuhn[1], Vicente Martinez[1], Scott B Evans[1], Nephi Stella[1,2] & Paul E M Phillips[1,2]

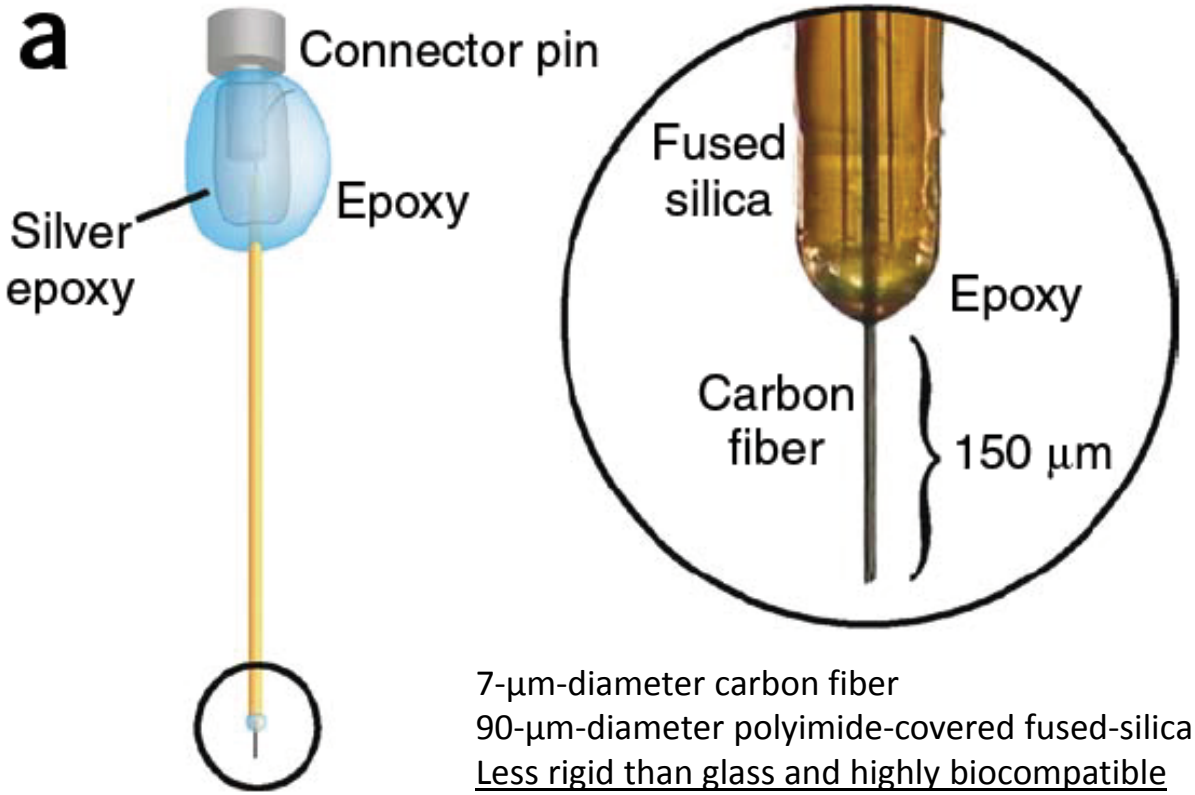
126 | VOL.7 NO.2 | FEBRUARY 2010 | NATURE METHODS



→ biocompatible voltammetric microsensor that can be chronically implanted

→ Detection of subsecond dynamics from day to day over periods of months

Image from Garris, Nature Methods, 2010

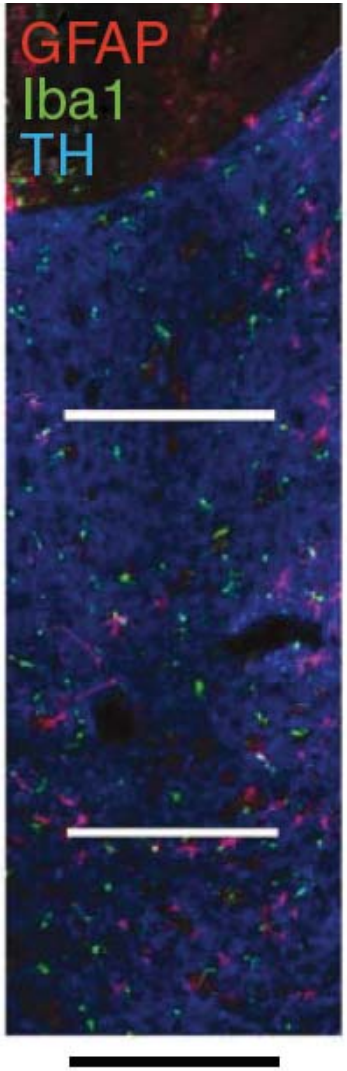


7-µm-diameter carbon fiber
90-µm-diameter polyimide-covered fused-silica capillary:
Less rigid than glass and highly biocompatible

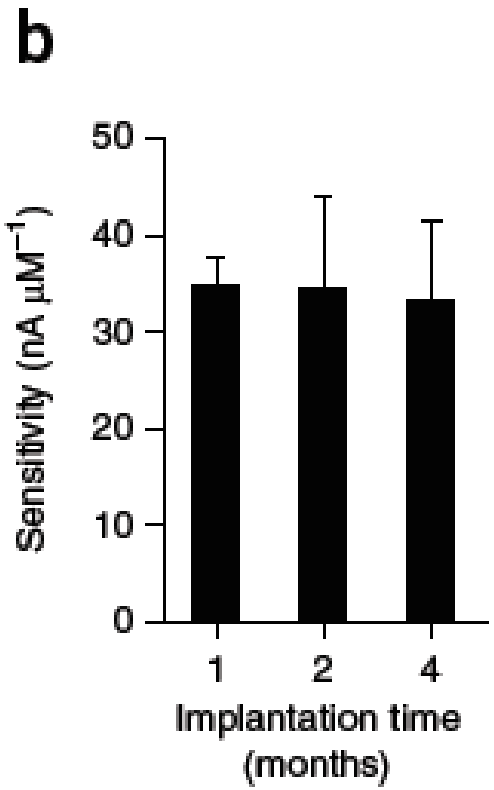
Figure 1 | Chronic carbon-fiber microsensor. (**a**) Schematic of the chronic microsensor, which consisted of a carbon fiber encased in a polyimide-fused silica. To ensure electric insulation, a two-component epoxy was applied to the fused silica–carbon fiber interface. At the opposite end, a female pin connector was electrically connected to the carbon fiber with silver epoxy. Finally, two-component epoxy was used to coat the connector for electrical insulation and structural integrity.

Clark et al., Nature Methods, 2010

Characterization in vitro after 1/2/4 months



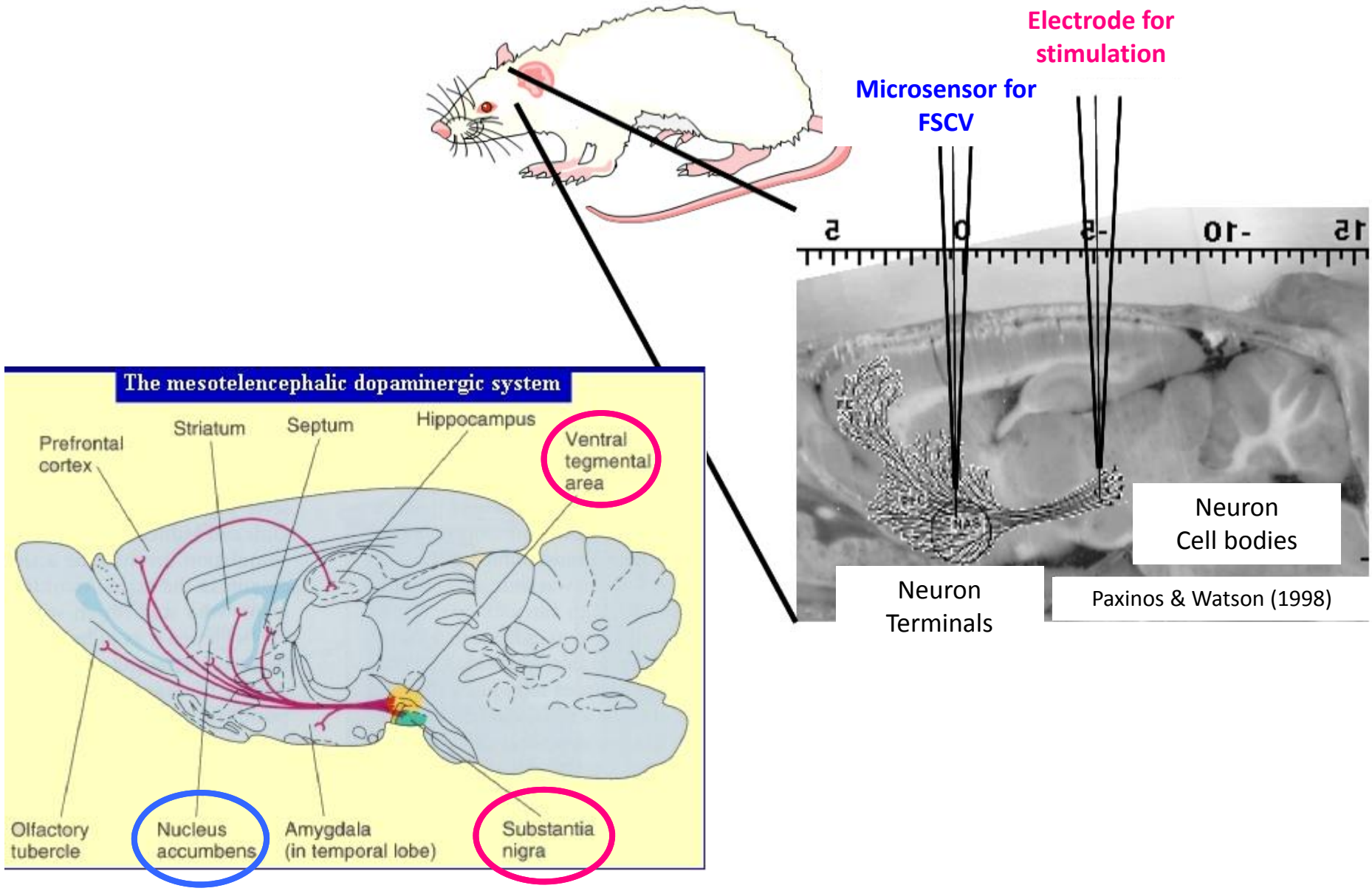
Rats implated for 10-16 weeks
Wide-field fluorescence image of a 30-µm striatal slice at a microsensor tract with no apparent gliosis present.
Scale bar, 300 µm.



Microsensor sensitivity to dopamine, assessed by flow injection analysis, after one (n = 5), two (n = 5) or four (n = 4) months of implantation

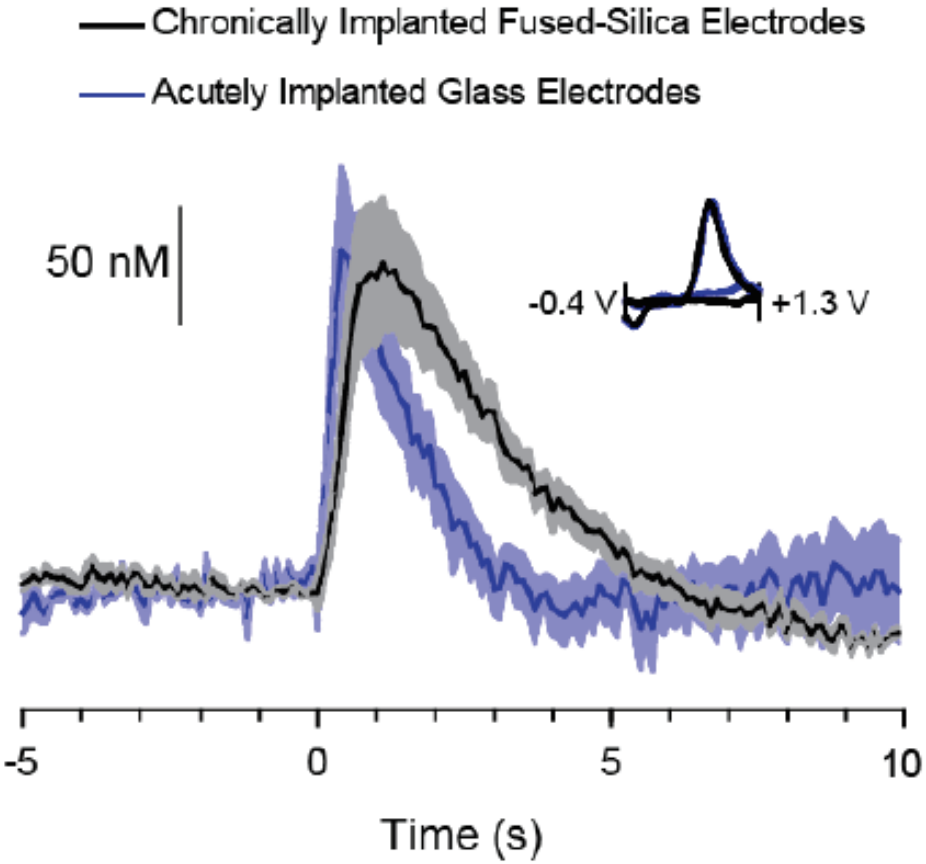
Clark et al., Nature Methods, 2010

In vivo settings



www4.ncsu.edu

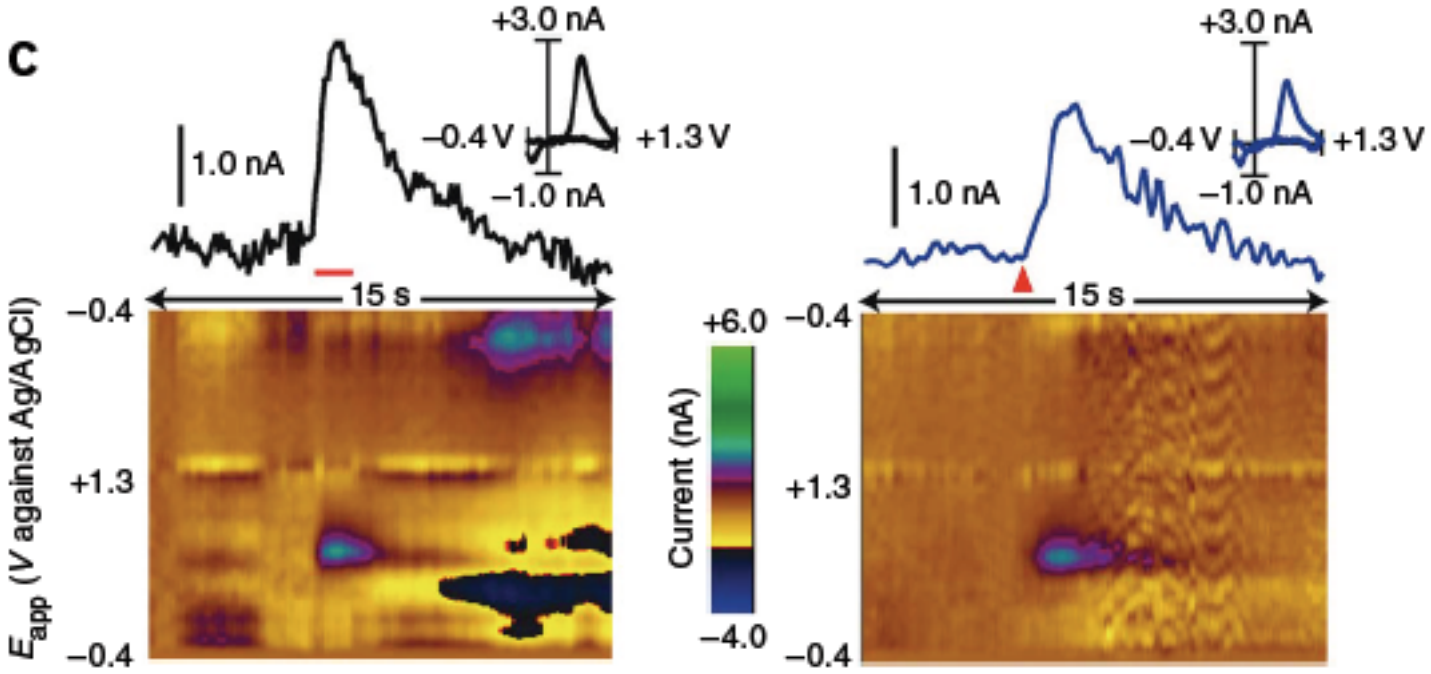
Chronically *vs* Acutely implanted electrode



electrically evoked dopamine release in the nucleus accumbens
comparable result, but temporal distortion

Clark et al., Nature Methods, 2010

Electrically *vs* reward induced dopamine release

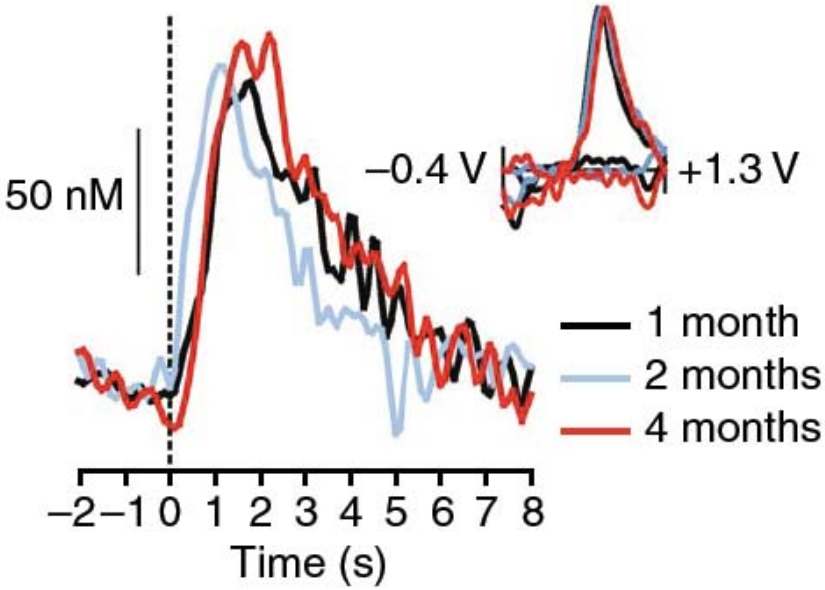


electrically evoked
dopamine release

food evoked
dopamine release

Clark et al., Nature Methods, 2010

Characterization in vivo after 1/2/4 months



reward-evoked dopamine release in the nucleus accumbens 1, 2 and 4 months after implantation.

Can it be combined with behavioural analysis over multiple days?

Clark et al., Nature Methods, 2010

Classical conditioning / Pavlovian conditioning

→ form of learning in which the ***conditioned stimulus*** (**CS**), comes to signal the occurrence of a second stimulus, the ***unconditioned stimulus*** (**US**)

Conditioning {  **CS** **+**  **US**

→ conditioned <u>cue-reward pairings increase neuronal firing in midbrain neurons</u>

→ phasic dopamine release events in response to primary rewards and their predictors

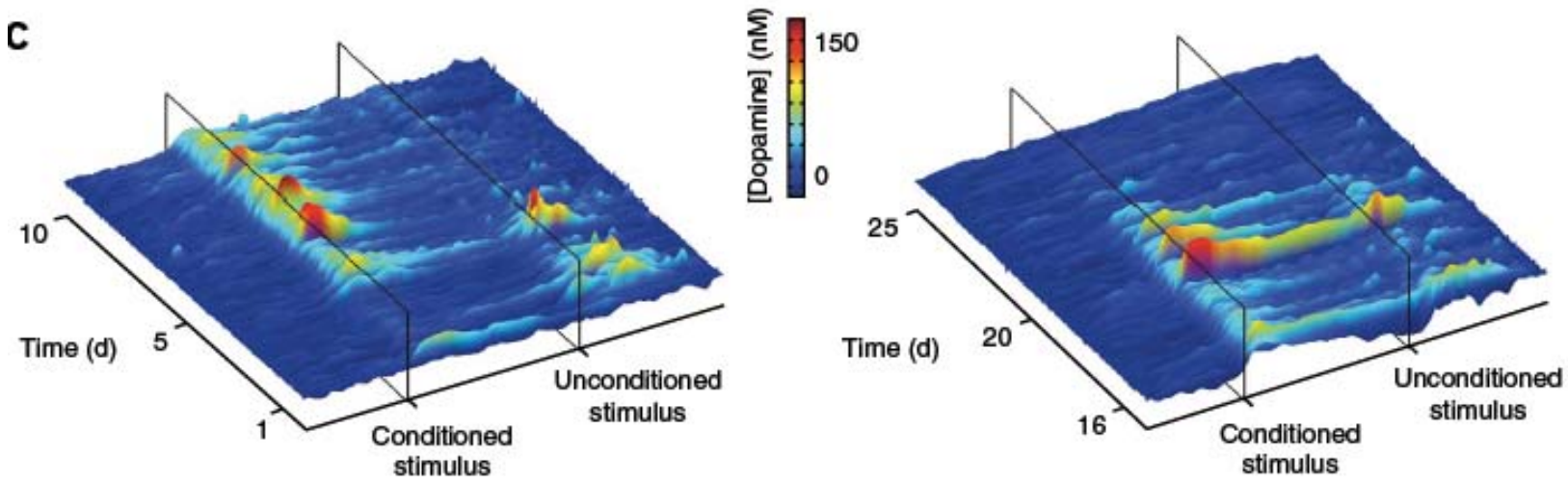
→ No data on dopamine release throughout learning

Longitudinal FSCV and behavioural analyses

Trial: 8-s presentation of a **light cue** (conditioned stimulus) followed immediately by the delivery of a **food pellet** (unconditioned stimulus).

Training session: 25 trials/day presented on a 60-s variable-interval schedule (tot= 25 days)

After 15 days of standard training:
<u>reward value increased</u> from 1 to 4 food pellets for 5 d followed by
5 d of <u>extinction training</u> during which food reward was omitted.



Clark et al., Nature Methods, 2010

LETTER

doi:10.1038/nature12475

Prolonged dopamine signalling in striatum signals proximity and value of distant rewards

Mark W. Howe[1], Patrick L. Tierney[1], Stefan G. Sandberg[2], Paul E. M. Phillips[2] & Ann M. Graybiel[1]

[1]McGovern Institute for Brain Research and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA. [2]Department of Psychiatry and Behavioral Sciences, and Department of Pharmacology, University of Washington, Seattle, Washington 98195, USA.

Methods

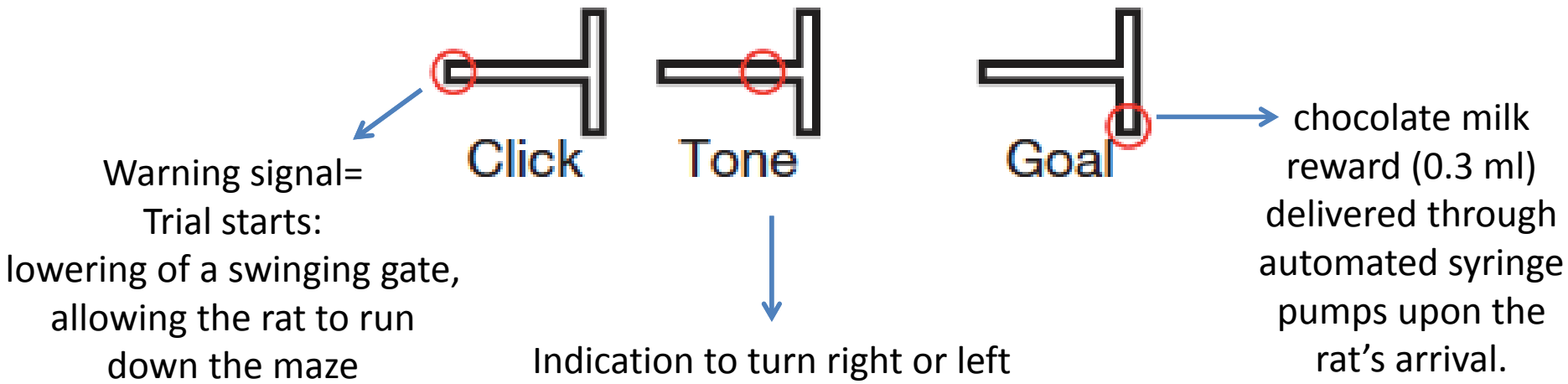
Male Long Evans rats

Voltammetry microsensors surgically implanted in nucleus accumbens

Training in T-maze: started 4 weeks after surgery, continued 15 to 35 days for each rat

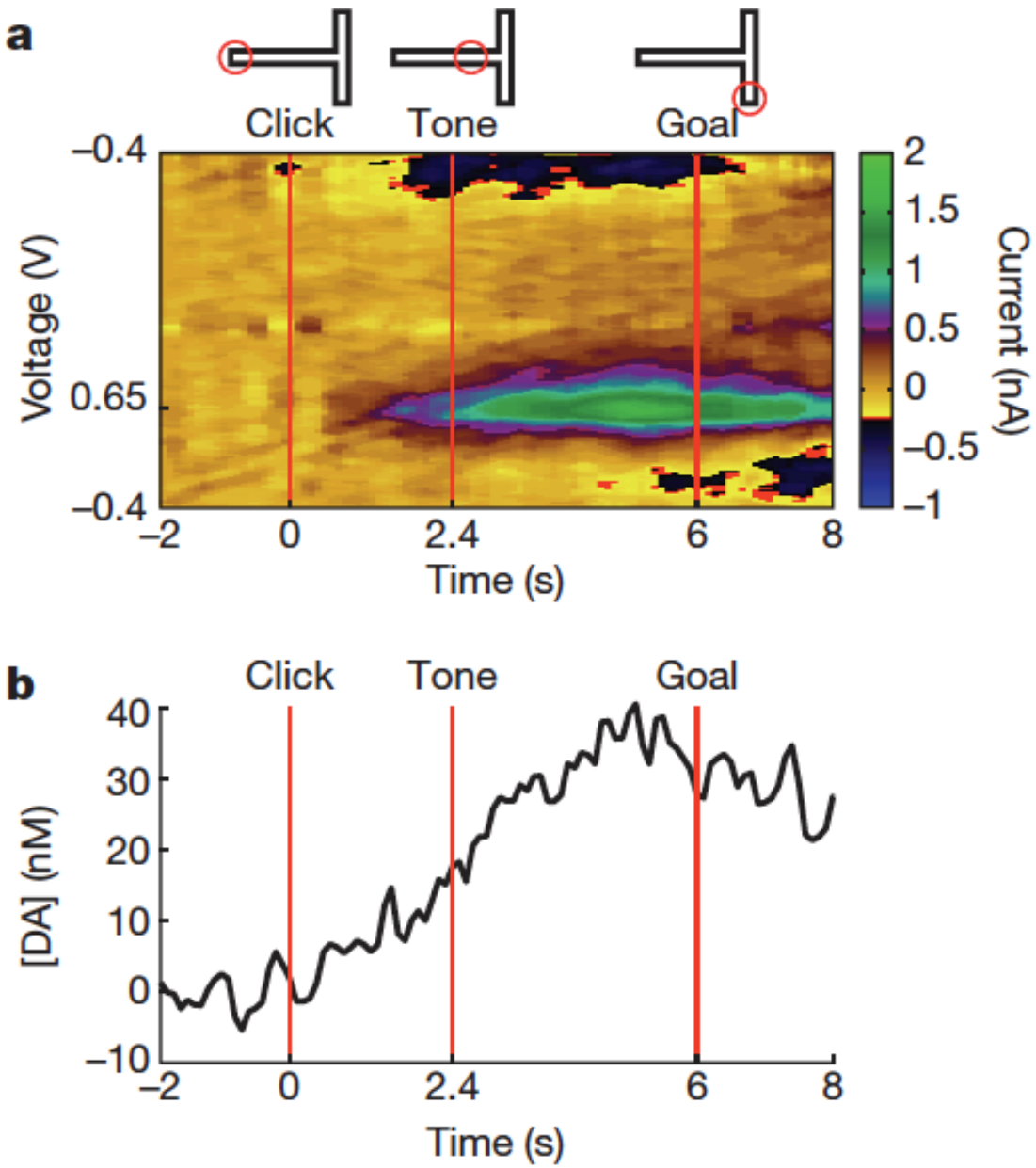
Daily behavioural sessions consisted of 40 trials

Voltammetric recordings began when rats learned to run smoothly down the track to retrieve reward. The spatial position of each rat was monitored continually by video tracking



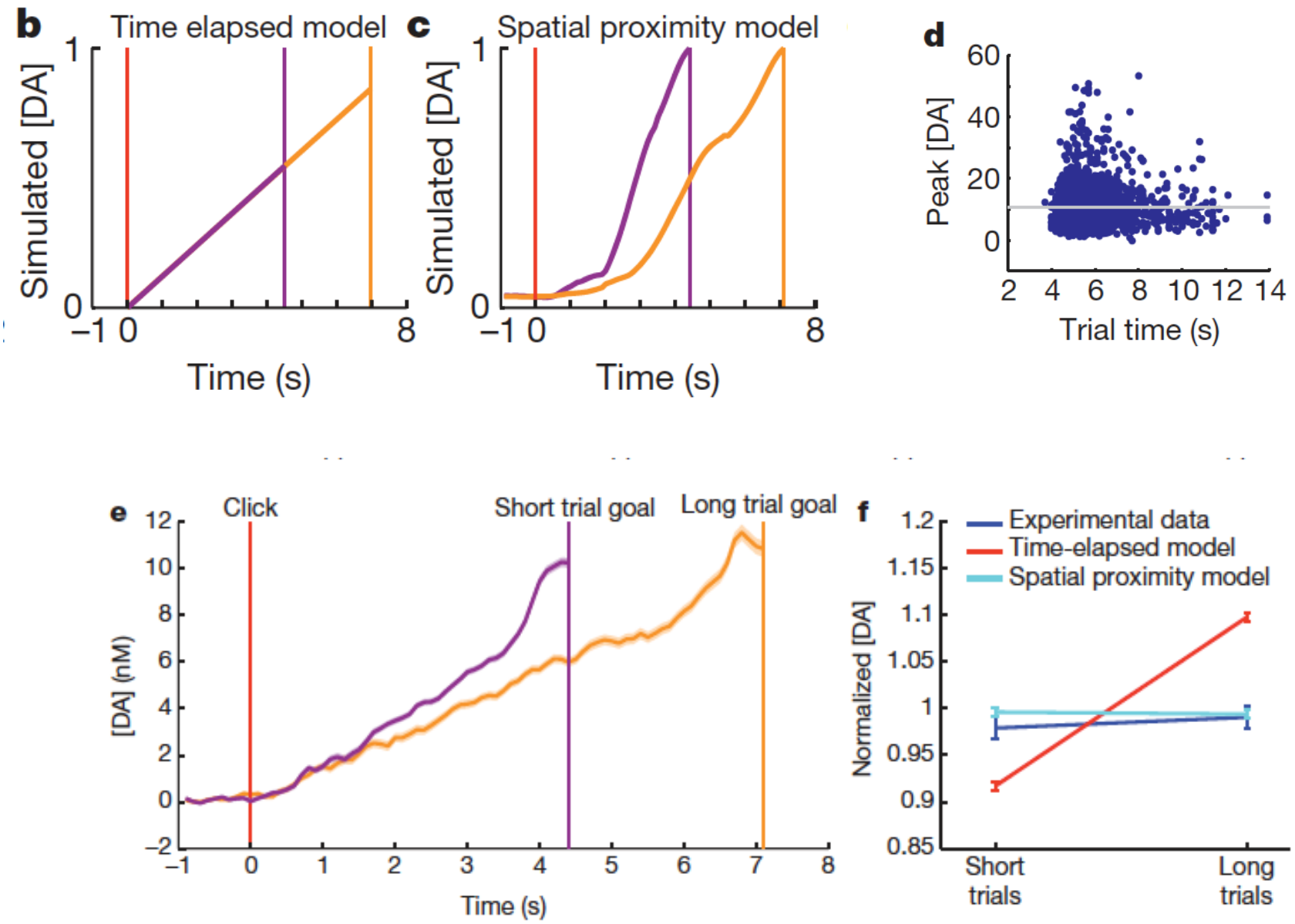
Howe et al., Nature, 2013

Dopamine ramps during maze runs



Howe et al., Nature, 2013

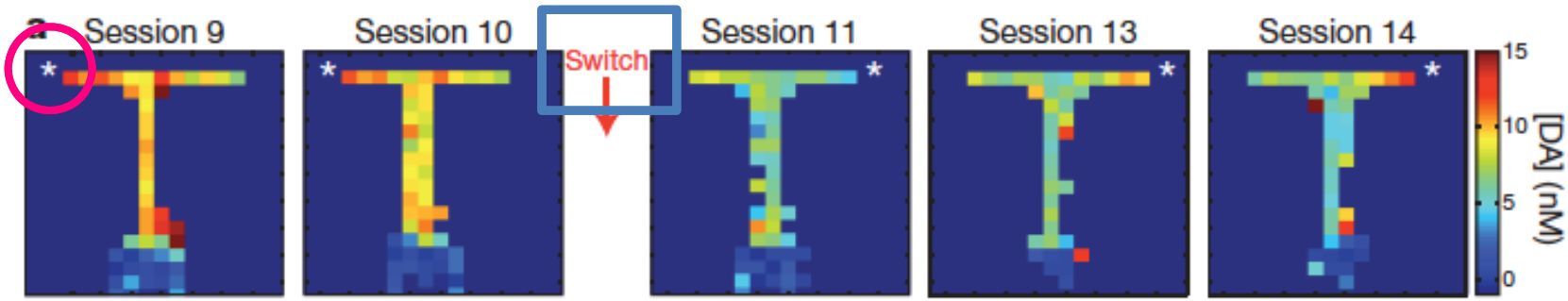
Does dopamine ramp as a function of time or proximity?

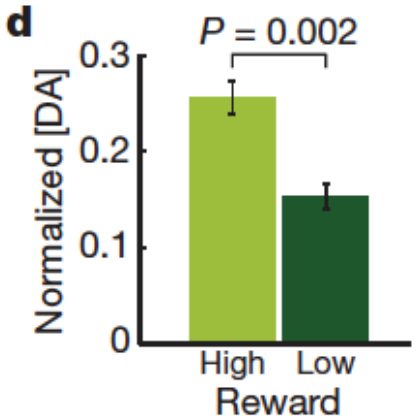


Howe et al., Nature, 2013

More reward = more dopamine

Asterisk indicates larger reward → higher levels DA for larger reward





Dopamine levels depend on the relative reward value

Howe et al., Nature, 2013

NEUROSCIENCE

Dopamine ramps up

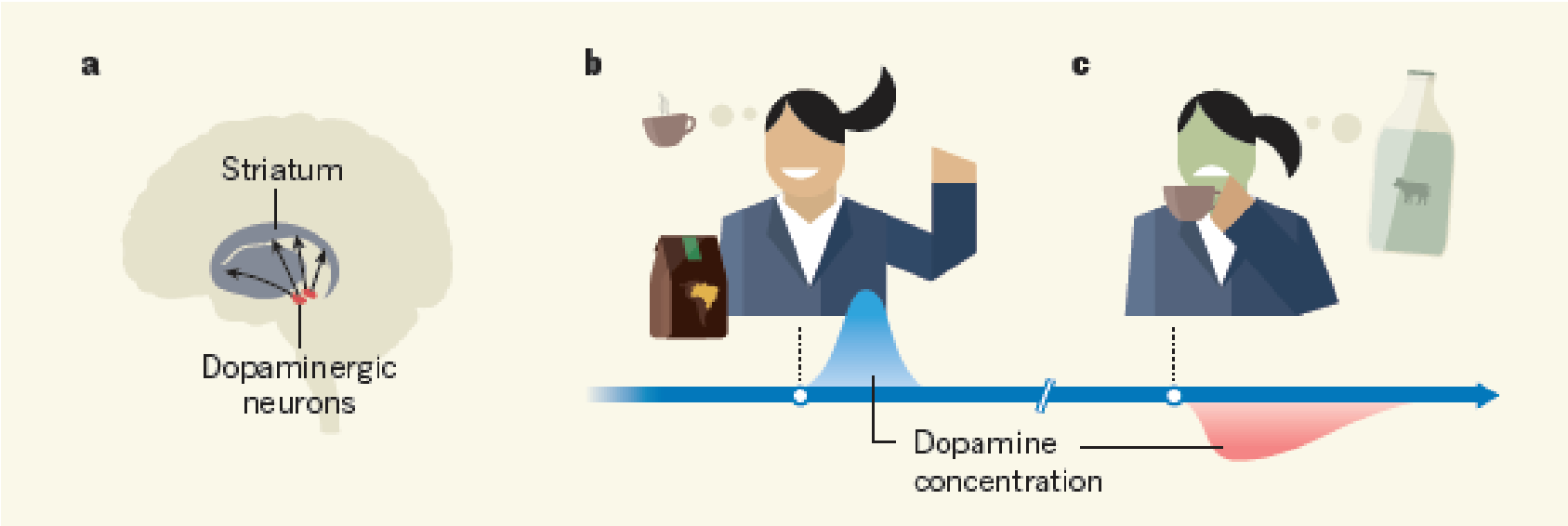
We thought we had figured out dopamine, a neuromodulator involved in everything from learning to addiction. But the finding that dopamine levels ramp up as rats navigate to a reward may overthrow current theories. **SEE LETTER P.575**

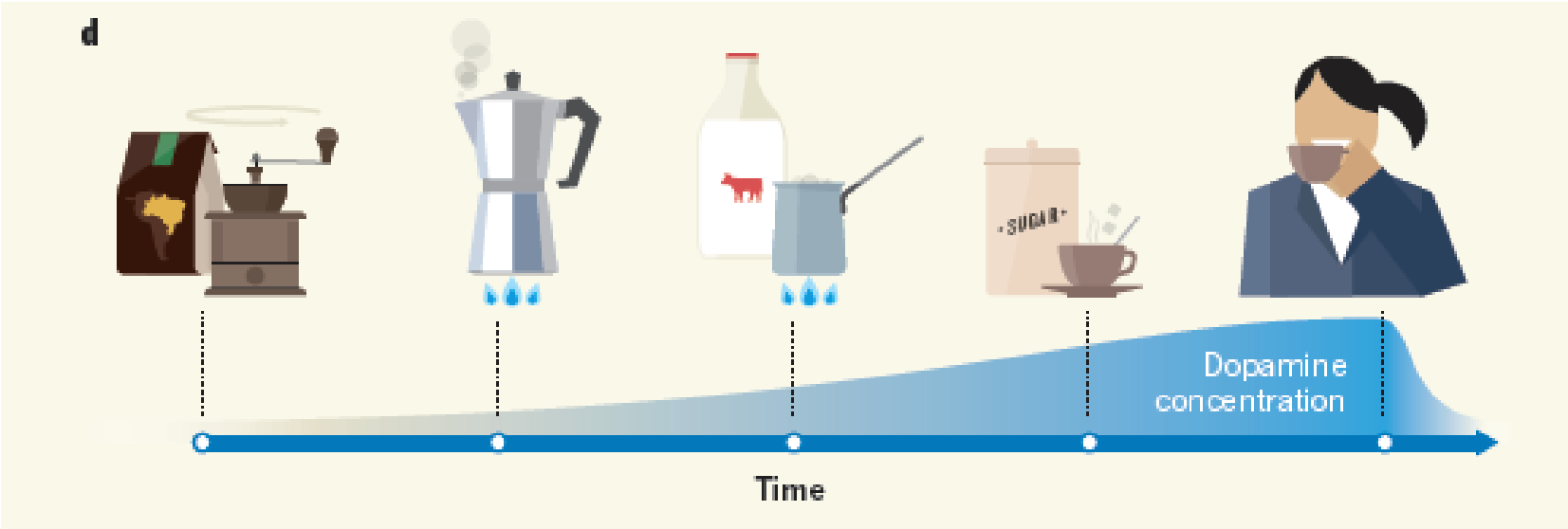
YAEL NIV

Scientific findings typically come in two flavours: explanations for things we already knew occurred but had no idea why, or new phenomena that are clearly important but still mysterious. Howe and colleagues' finding[1], on page 575 of this issue, is of the latter kind — even if we don't yet know what it means, it stands to alter the way we think about dopamine.

striatum of rats while the animals ran through mazes for food rewards. The striatum (Fig. 1a) is the area that contains the highest dopamine concentration in the brain. It is involved in action selection at all levels, from choosing which limb to move to selecting a goal to work towards. In a series of elegant experiments, Howe *et al.* established that dopamine concentration gradually ramps up as rats run towards a reward, and that the slope of the ramps relates to the amount of anticipated reward and the

Reward and the brain





Dopamine acts to maintain and direct motivational resources during prolonged behaviour

Niv, Nature, 2013

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Cell-based neurotransmitter fluorescent engineered reporters (CNiFERs)

nature neuroscience

TECHNICAL REPORT

An *in vivo* biosensor for neurotransmitter release and *in situ* receptor activity

Quoc-Thang Nguyen[1,7], Lee F Schroeder[2,3,7], Marco Mank[4], Arnaud Muller[1], Palmer Taylor[5], Oliver Griesbeck[4] & David Kleinfeld[1,3,6]

[1]Physics Department, [2]Medical Scientist Training Program, [3]Graduate Program in Neurosciences, University of California San Diego, La Jolla, California, USA. [4]Max-Planck Institut für Neurobiologie, Martinsried, Germany. [5]Skaggs School of Pharmacy and Pharmaceutical Sciences, [6]Center for Neural Circuits and Behavior, University of California San Diego, La Jolla, California, USA. [7]These authors contributed equally to this work. Correspondence should be addressed to D.K. (dk@physics.ucsd.edu).

Received 25 August; accepted 17 November; published online 13 December 2009; doi:10.1038/nn.2469

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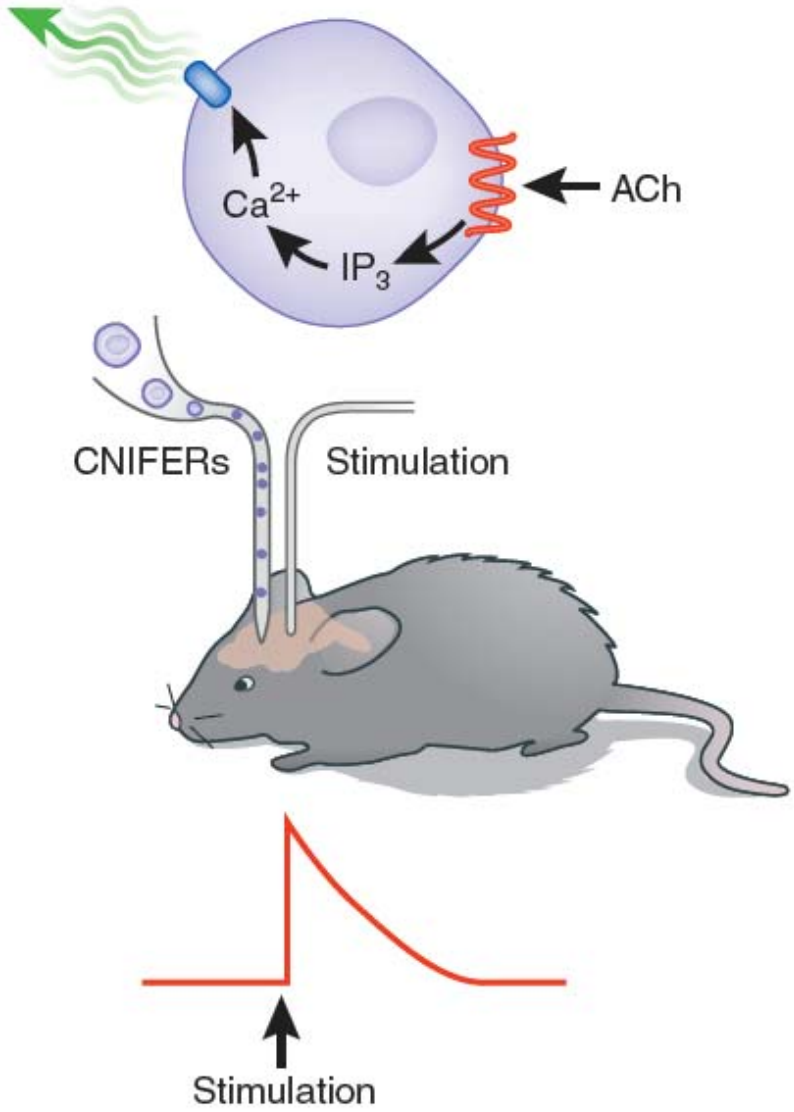
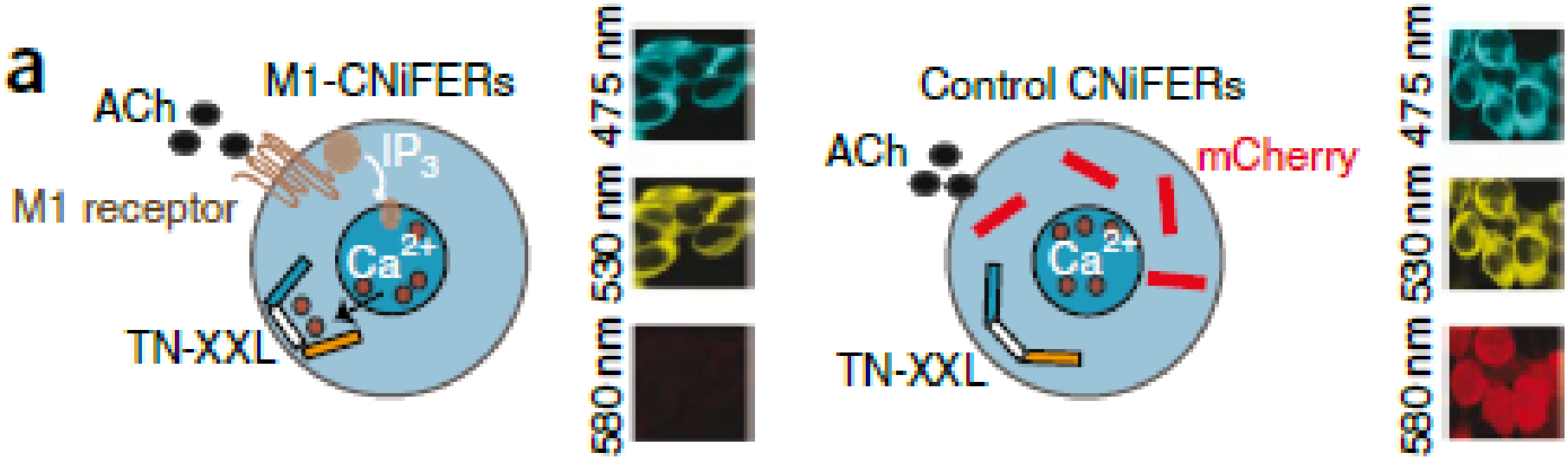


Image from Garris, Nature Methods, 2010

Generation of M1-CNiFERs



HEK293 cells transduced to express the **M1 receptor**, a major muscarinic receptor in neocortex, + **calcium indicator TN-XXL15**

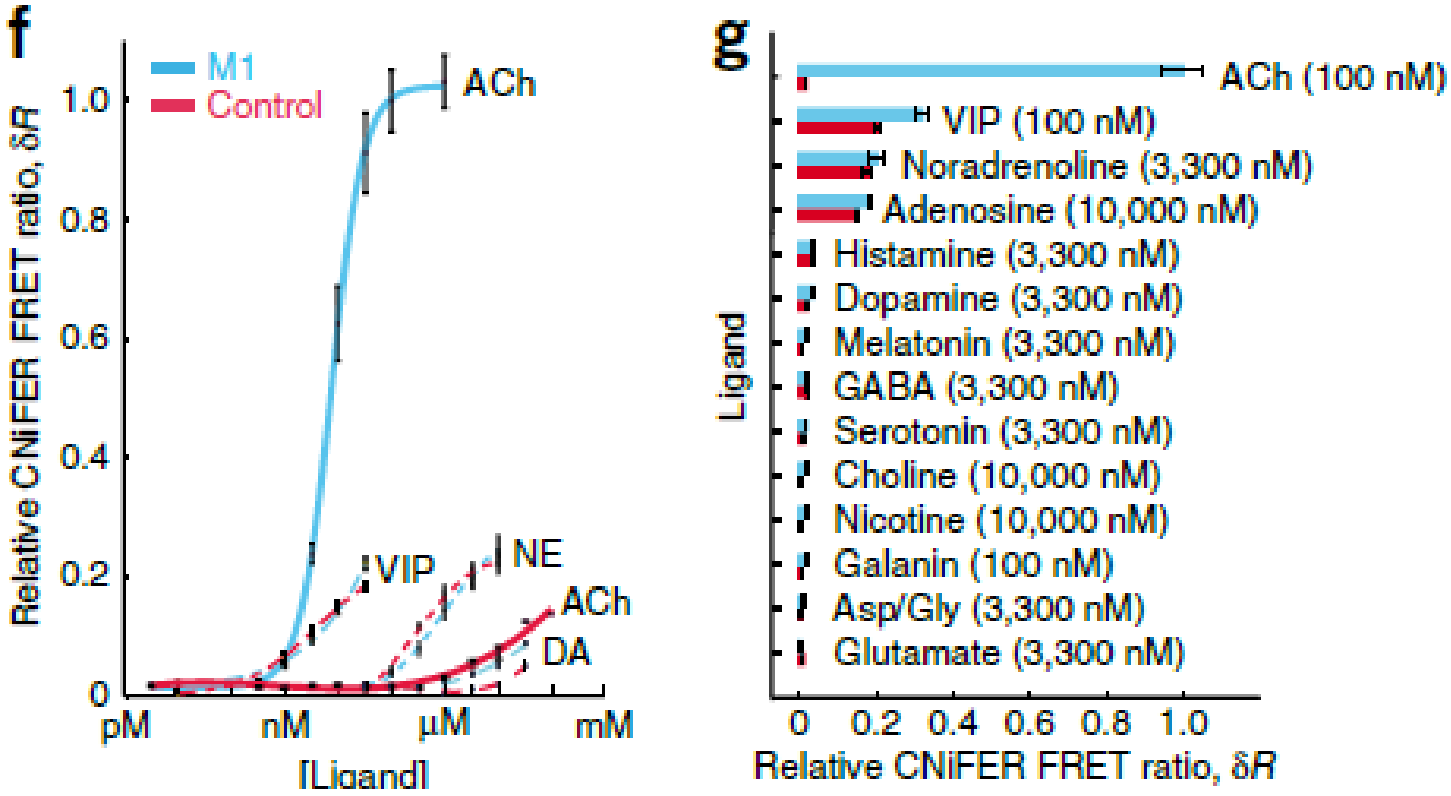
When M1 is activated → Gq/IP3 pathway → Ca^{2+} increase → binding to TN-XXL →

FRET: decrease in cyan increase in yellow fluorescence

Nguyen et al., Nature Neuroscience, 2010

Specificity of M1 CNiFERs in vitro

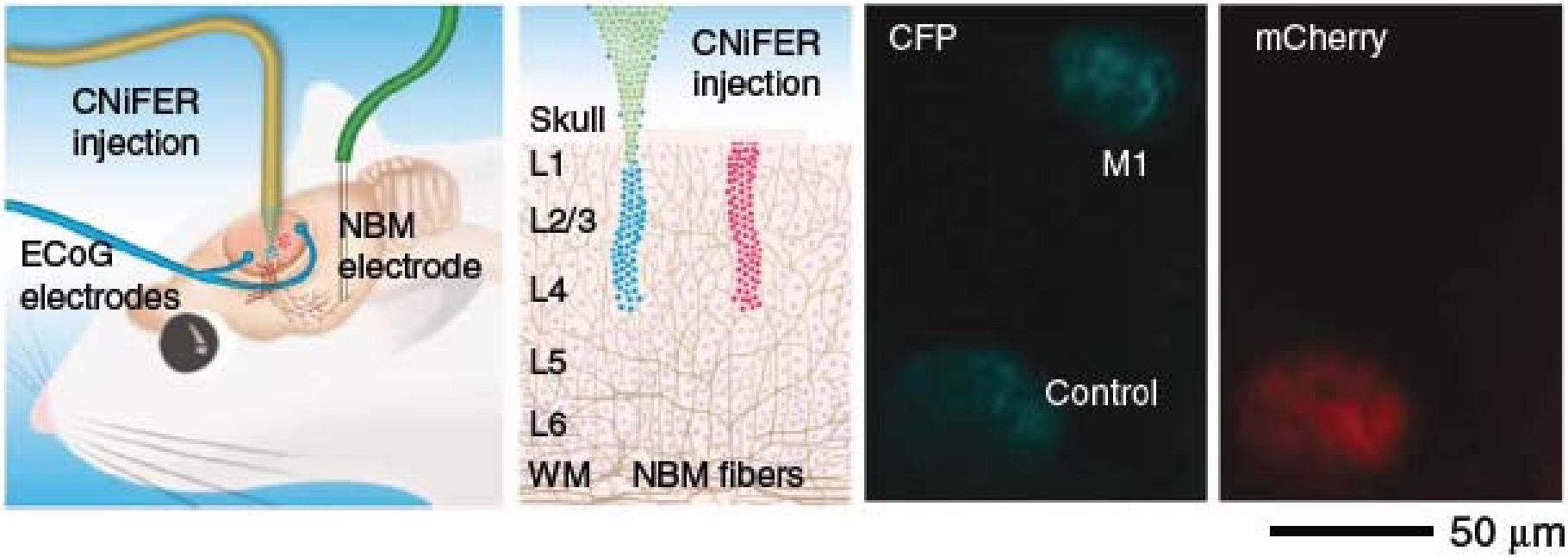
!!! HEK293 cells can express endogenous surface receptors.



Potentially confounding neurotransmitters elicited signals only at higher concentrations and/or activated control CNiFERs

Nguyen et al., Nature Neuroscience, 2010

Implantation of CNiFERs in vivo



CNiFERs were implanted in rat frontal cortex: 10–30 CNiFERs per site (M1 and Ctr)

Stimulating electrodes were implanted in NBM (nucleus basalis magnocellularis), a basal forebrain structure that projects cholinergic fibers into neocortex

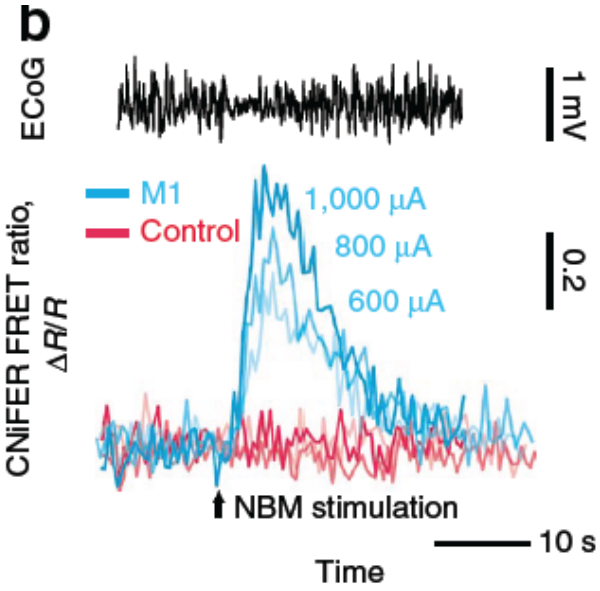
Electrocorticogram (ECoG) wires were placed to detect NBM-evoked cortical activation

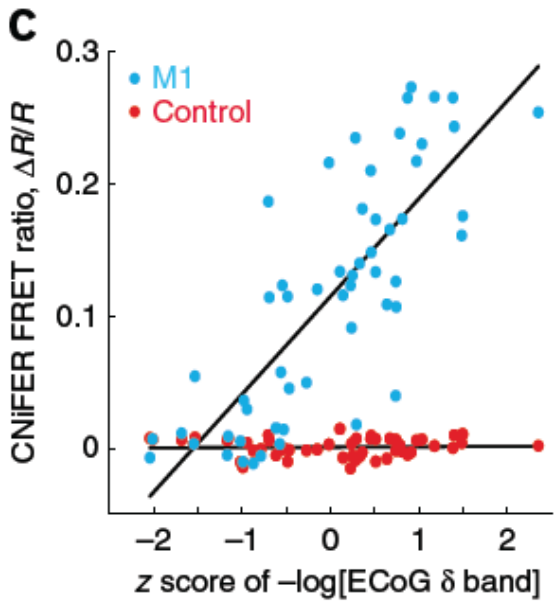
two-photon laser-scanning microscopy down to 300 µm below the cortical surface

Nguyen et al., Nature Neuroscience, 2010

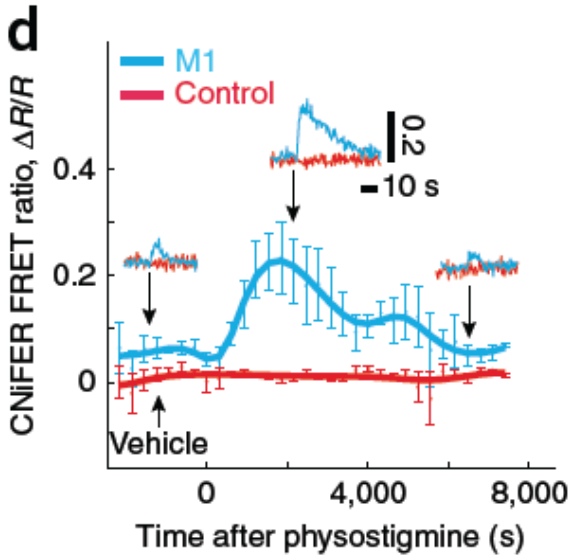
In vivo characterization of acutely implanted M1-CNiFERs

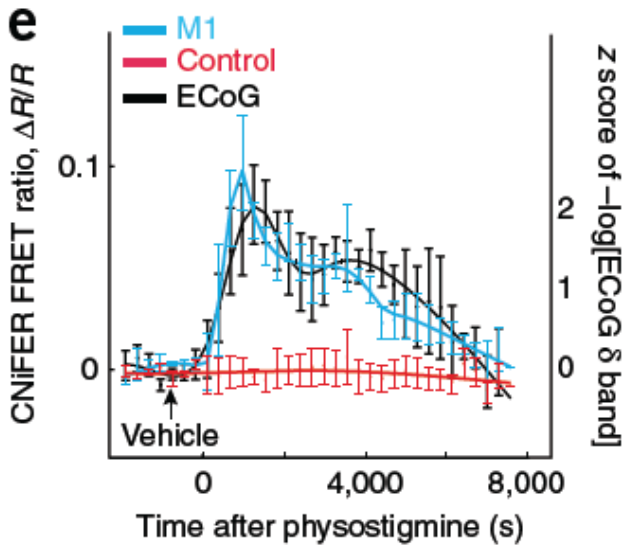
Single train excitation of NBM





NMB stimulation every 300s + s.c. injection of physostigmine, an acetylcholinesterase inhibitor → enhanced the amplitude and duration of the NBM-evoked M1-CNiFER response





s.c. injection of physostigmine

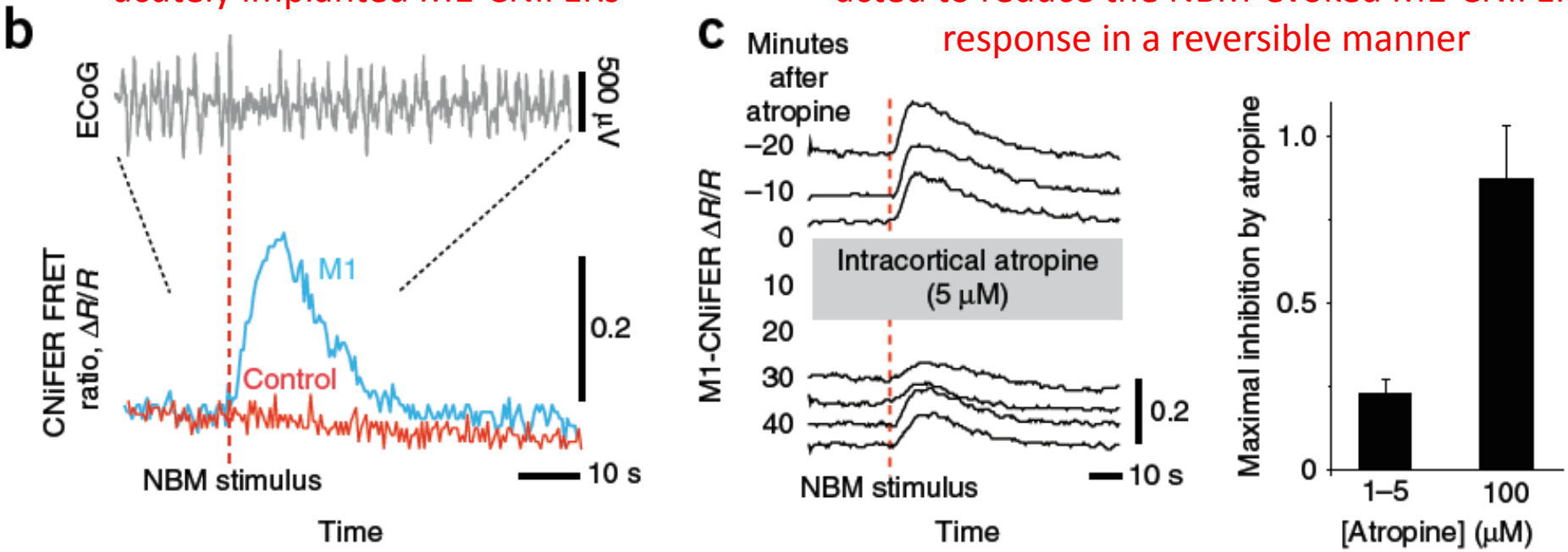
Nguyen et al., Nature Neuroscience, 2010

Chronic implantation of CNiFERs

Chronically implanted M1-CNiFERs can be imaged for at <u>least 6 d</u>

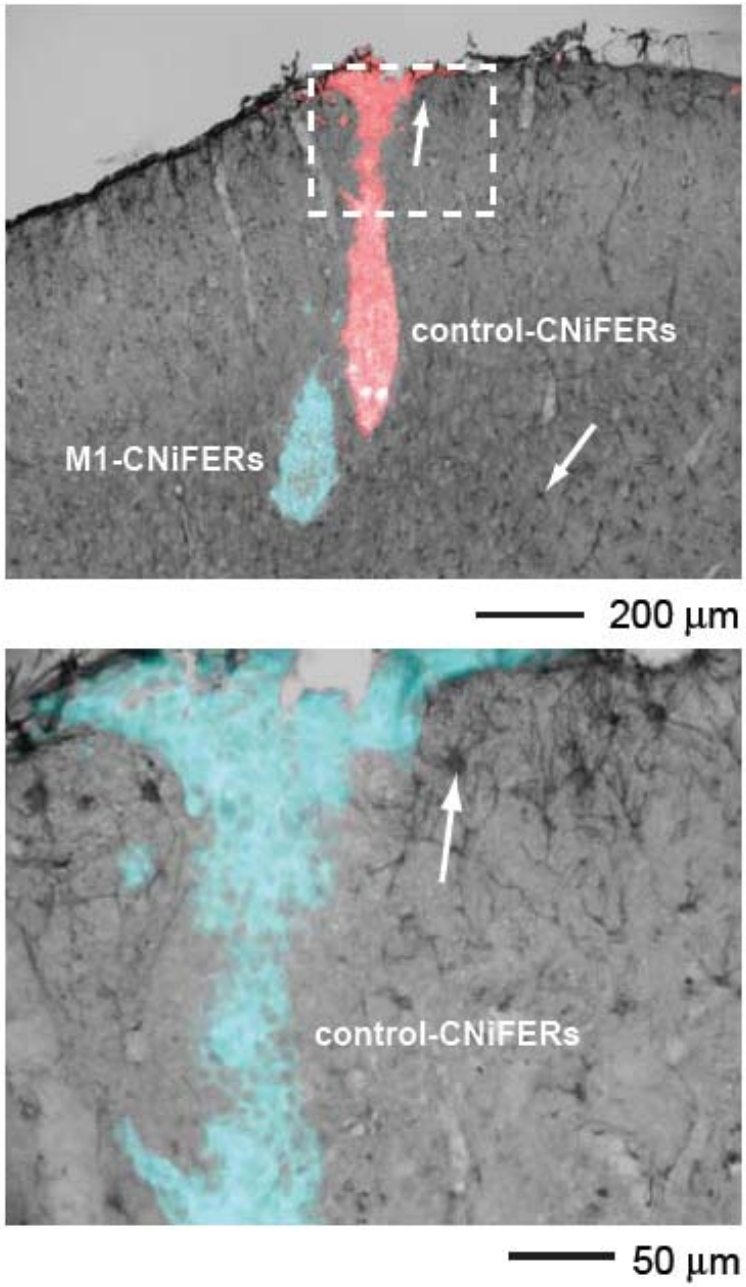
response was very similar to that found with acutely implanted M1-CNiFERs

5 μM atropine near the site of implantation acted to reduce the NBM-evoked M1-CNiFER response in a reversible manner



Nguyen et al., Nature Neuroscience, 2010

Chronic implantation of CNiFERs



minimal tissue damage, negligible presence of reactive astrocytes and no evidence for intracortical cell proliferation (Images 2d after implantation)

Nguyen et al., Nature Neuroscience, 2010

Cell-based reporters reveal *in vivo* dynamics of dopamine and norepinephrine release in murine cortex

Arnaud Muller[1,6], Victory Joseph[2,6], Paul A Slesinger[2,3] & David Kleinfeld[1,2,4,5]

[1]Department of Physics, University of California at San Diego, La Jolla, California, USA. [2]Graduate Program in Neurosciences, University of California at San Diego, La Jolla, California, USA. [3]Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, New York, USA. [4]Section of Neurobiology, University of California at San Diego, La Jolla, California, USA. [5]Department of Electrical and Computer Engineering, University of California at San Diego, La Jolla, California, USA. [6]These authors contributed equally to this work. Correspondence should be addressed to D.K. (dk@physics.ucsd.edu) or P.A.S. (paul.slesinger@mssm.edu).

RECEIVED 22 APRIL; ACCEPTED 14 SEPTEMBER; PUBLISHED ONLINE 26 OCTOBER 2014; CORRECTED ONLINE 10 NOVEMBER 2014 (DETAILS ONLINE); DOI:10.1038/NMETH.3151

Background: neurotransmitters

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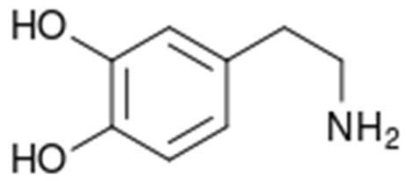
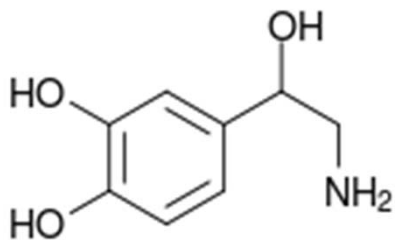
Norepinephrine	Tyrosine
Epinephrine	Tyrosine
Serotonin	Tryptophan
Histamine	Histidine
Melatonin	Serotonin
Amino acids	
Aspartate	Oxaloacetate
γ-Aminobutyric acid	Glutamine
Glutamate	Glutamine
Glycine	Serine
ATP	ADP
Adenosine	ATP
Arachidonic acid	Phospholipids
Carbon monoxide	Heme
Nitric oxide	Arginine

dopamine



dopamine
beta-hydroxylase

norepinephrine

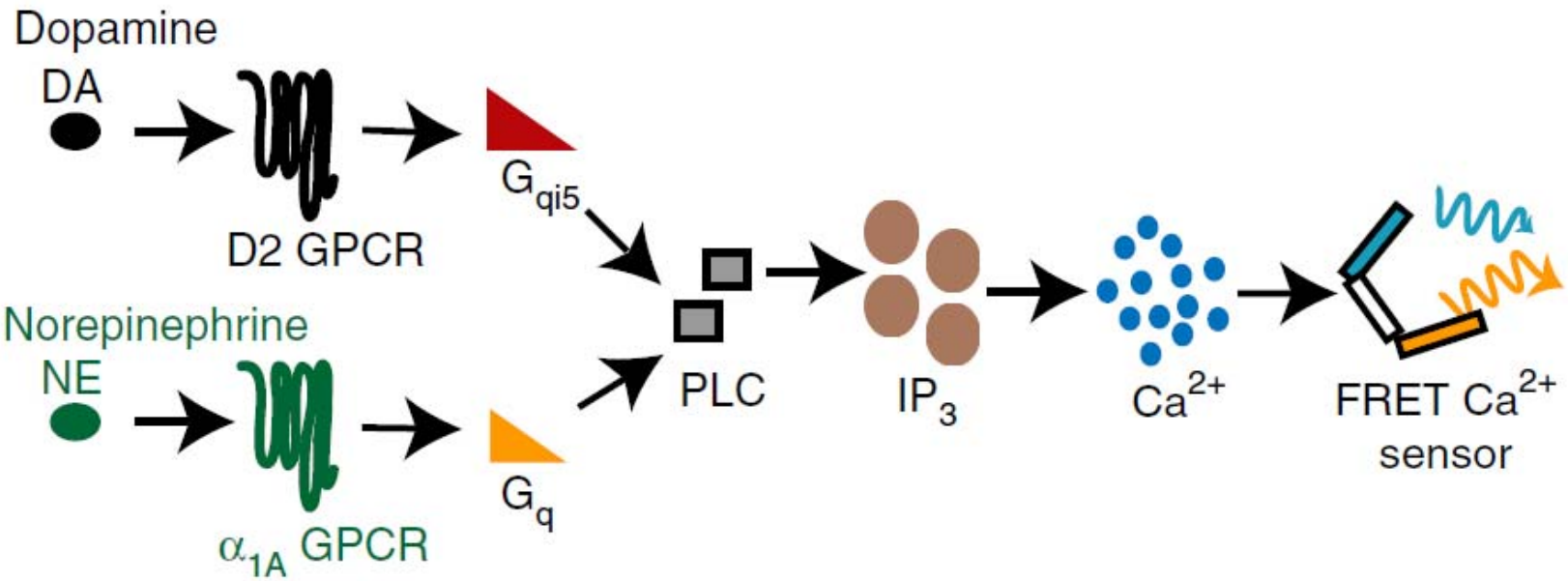


!! Indistinguishable cyclic voltammetry signatures

!! Cortex: inputs from dopaminergic and noradrenergic neurons

Kandel *et al.,* Principles of Neural Science, 5th edition, 2013

New CNiFERs to detect Dopamine (DA) and Norepinephrine (NE)

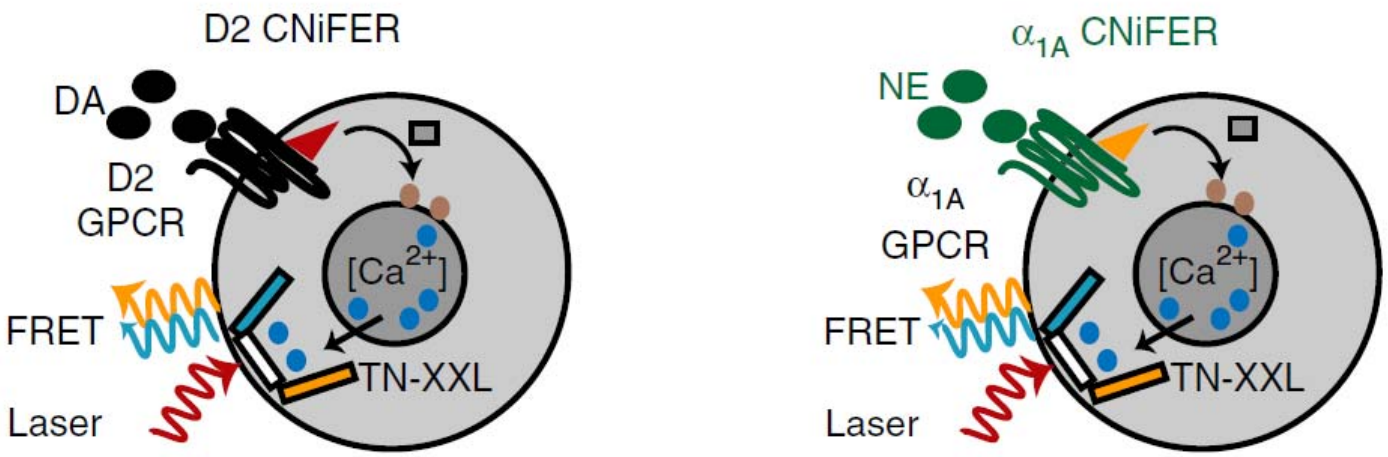


Dopamine → D_2 receptor → $G_{i/o}$ → new HEK293 with chimeric G_{qi5}: $G_{\alpha q}$ sequence for PLC + 5 amino acids of the $G_{i/o}$ for D_2

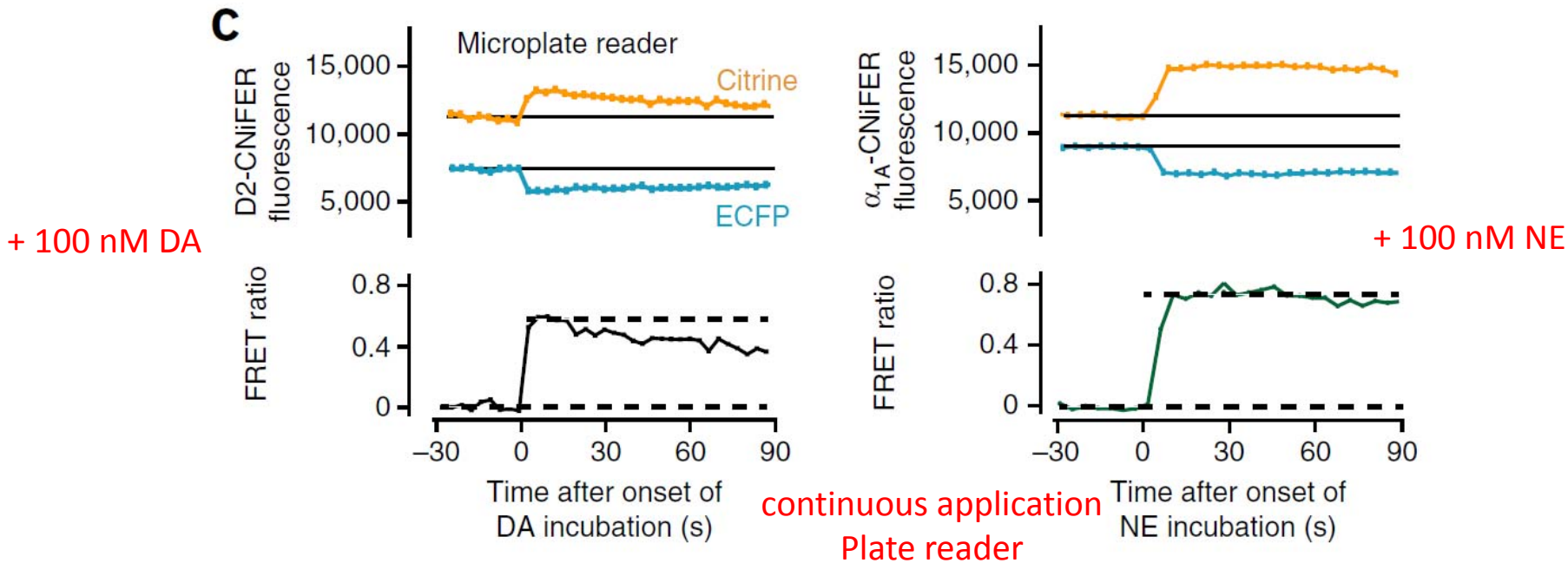
Norepinephrine → α_{1A} receptor → G_q

Muller *et al.,* Nature Methods, 2014

New CNiFERs to detect DA and NE: clone selection



Parent cells lacking the receptors were used as controls



Muller *et al.*, Nature Methods, 2014

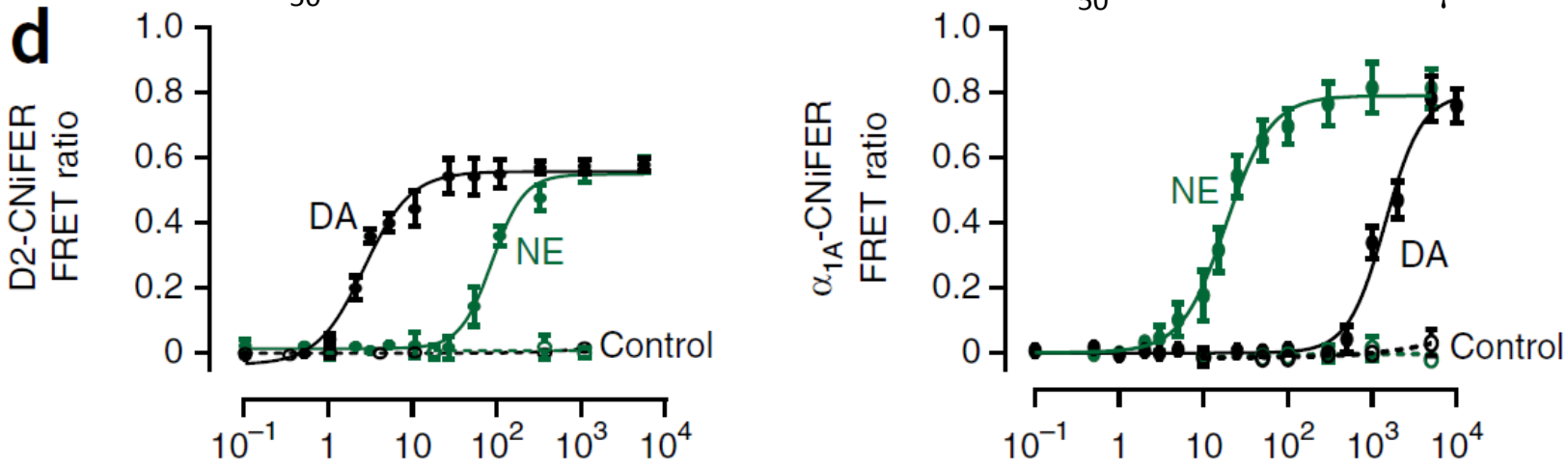
In vitro characterization: sensitivity and specificity (I)

EC_{50} for DA= 2.5 ± 0.1 nM

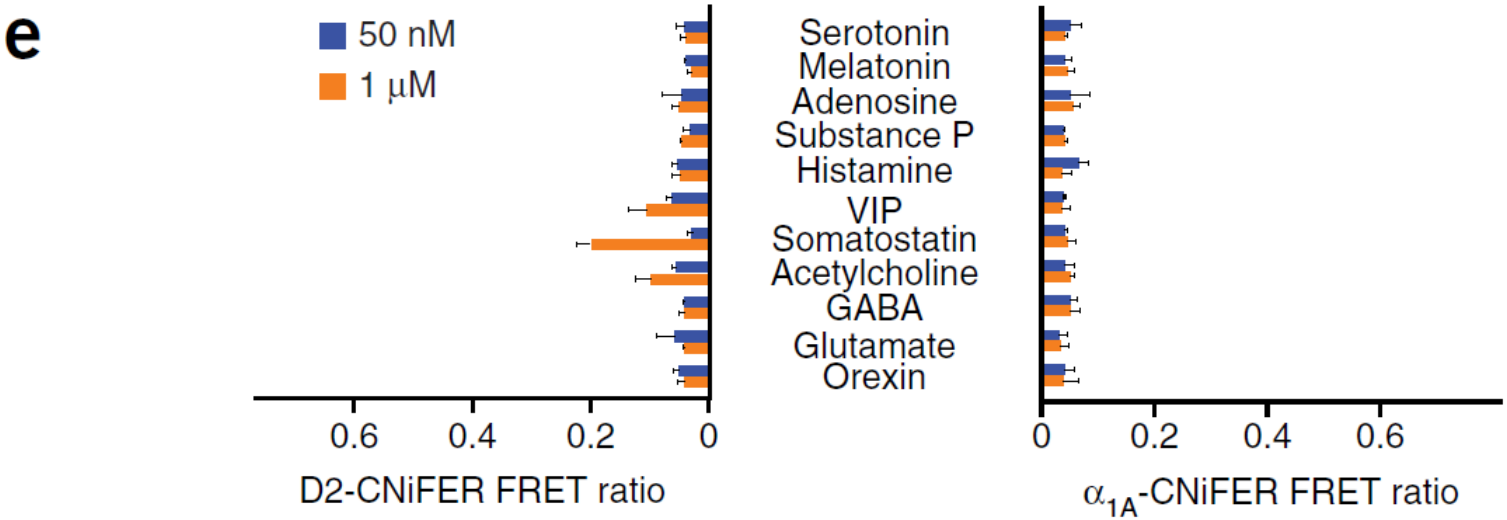
EC_{50} for NE= 81 ± 8 nM

EC_{50} for NE= 19 ± 1 nM

EC_{50} for NE= 1.4 ± 0.1 μM

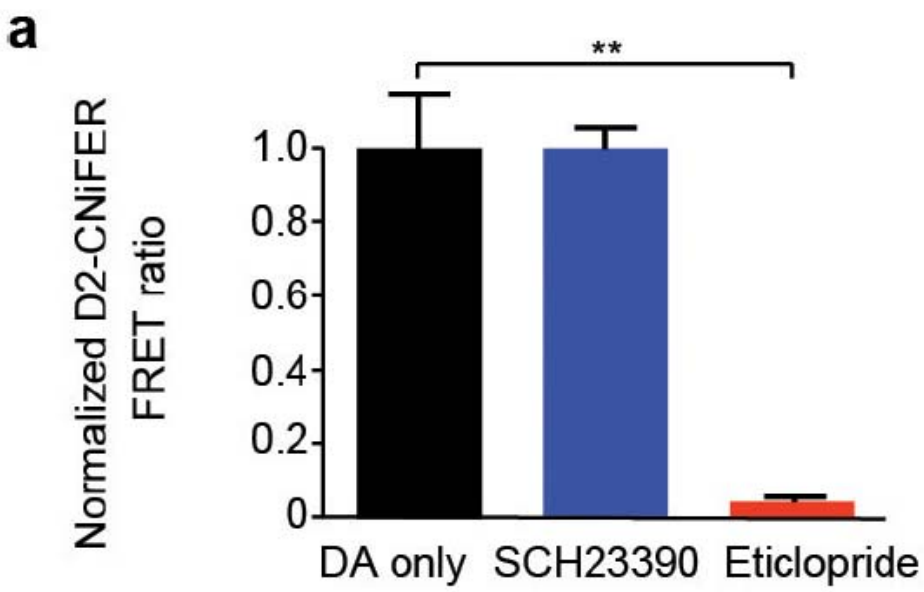


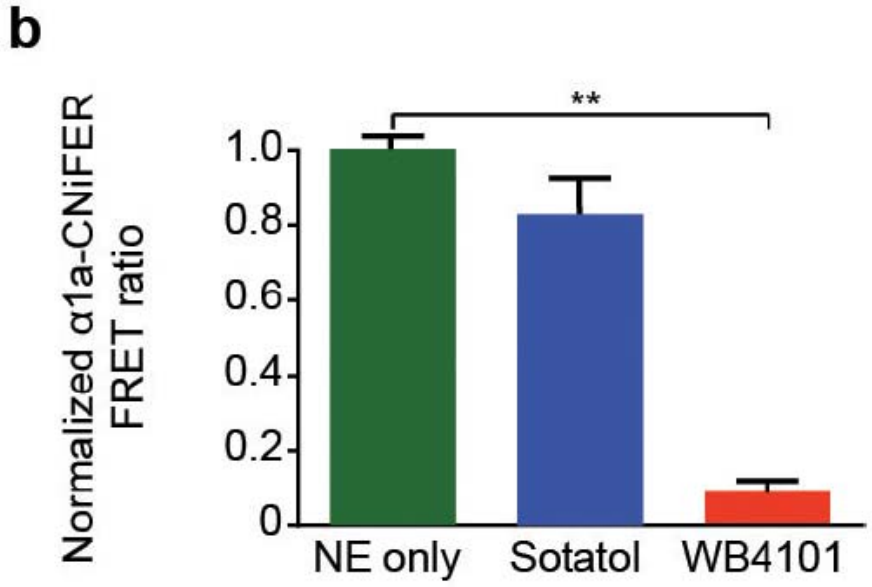
Specificity vs other neurotransmitters



Muller *et al.*, Nature Methods, 2014

In vitro characterization: specificity (II)





SCH23390 (100 nM)= D1-receptor antagonist
Eticlopride (50 nM)= D2-receptor antagonist

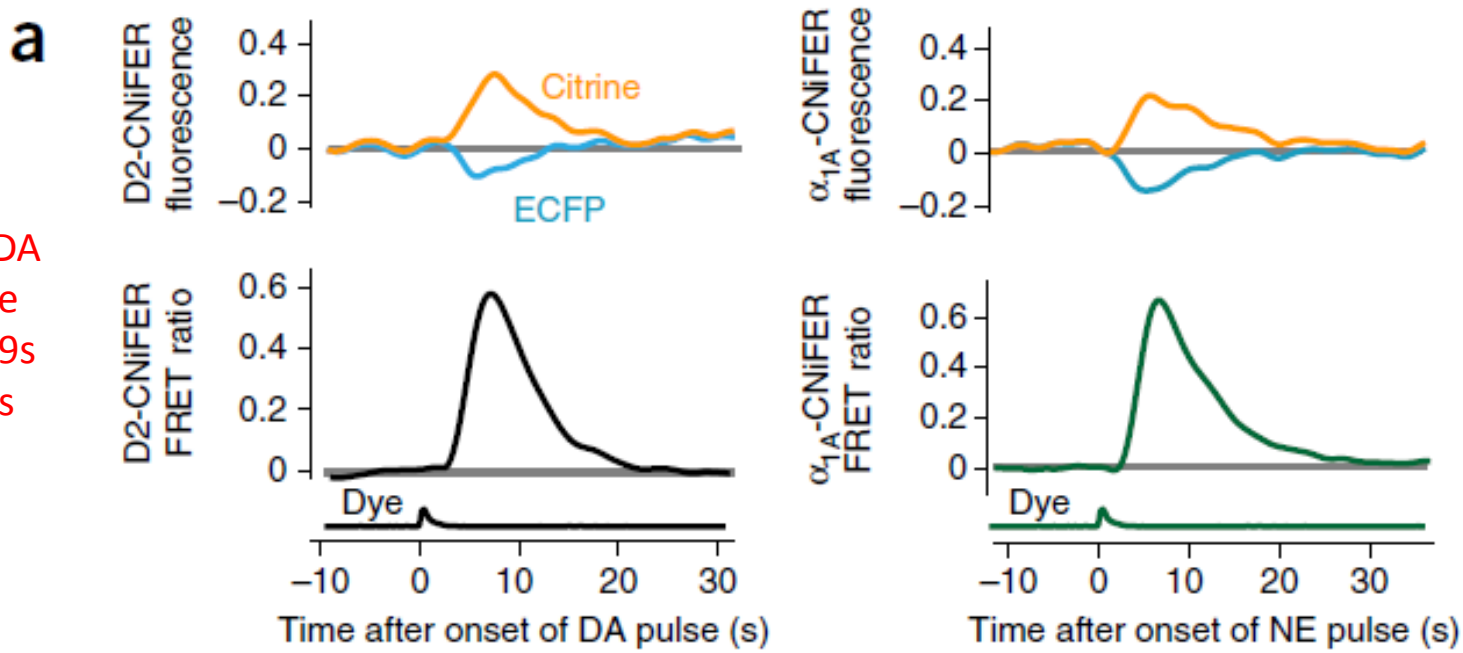
Sotatol (5 µM)= β-adrenergic–receptor antagonist
WB4101 (50 nM)= α1A-antagonist

Muller *et al.,* Nature Methods, 2014

In vitro characterization: response to agonist pulses (I)

2.5 s pulse application
Perfusion system
Cell clusters

100 nM DA
+ fluo dye
Delay=2.9s
Max=6.9s

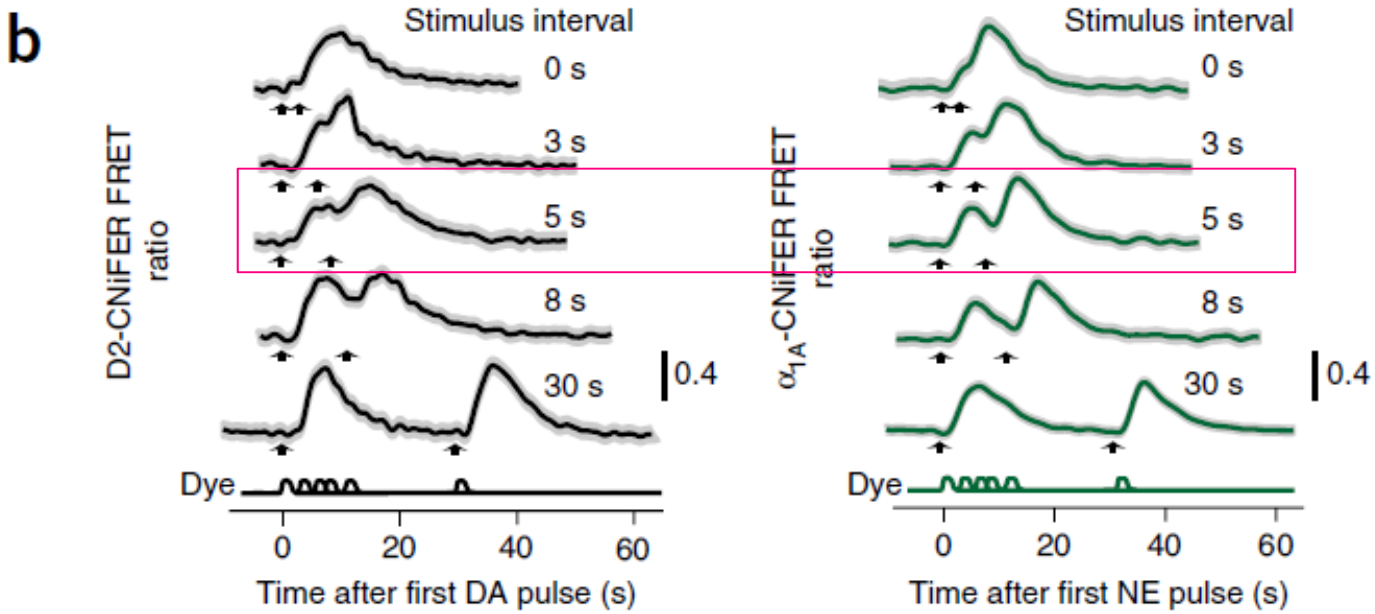


100 nM NE
+ fluo dye
Delay=2.3s
Max=5.1s

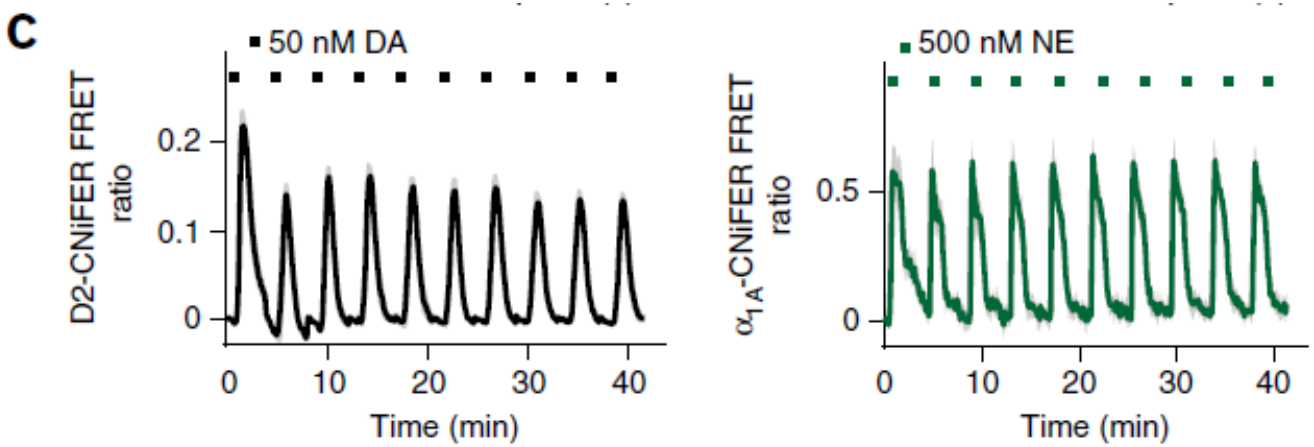
Signals returned to baseline after approx. 20s

Muller *et al.,* Nature Methods, 2014

In vitro characterization: response to agonist pulses (II)



Intermittent pulses to assess receptor desensitization: every 4 min, tot 40 min



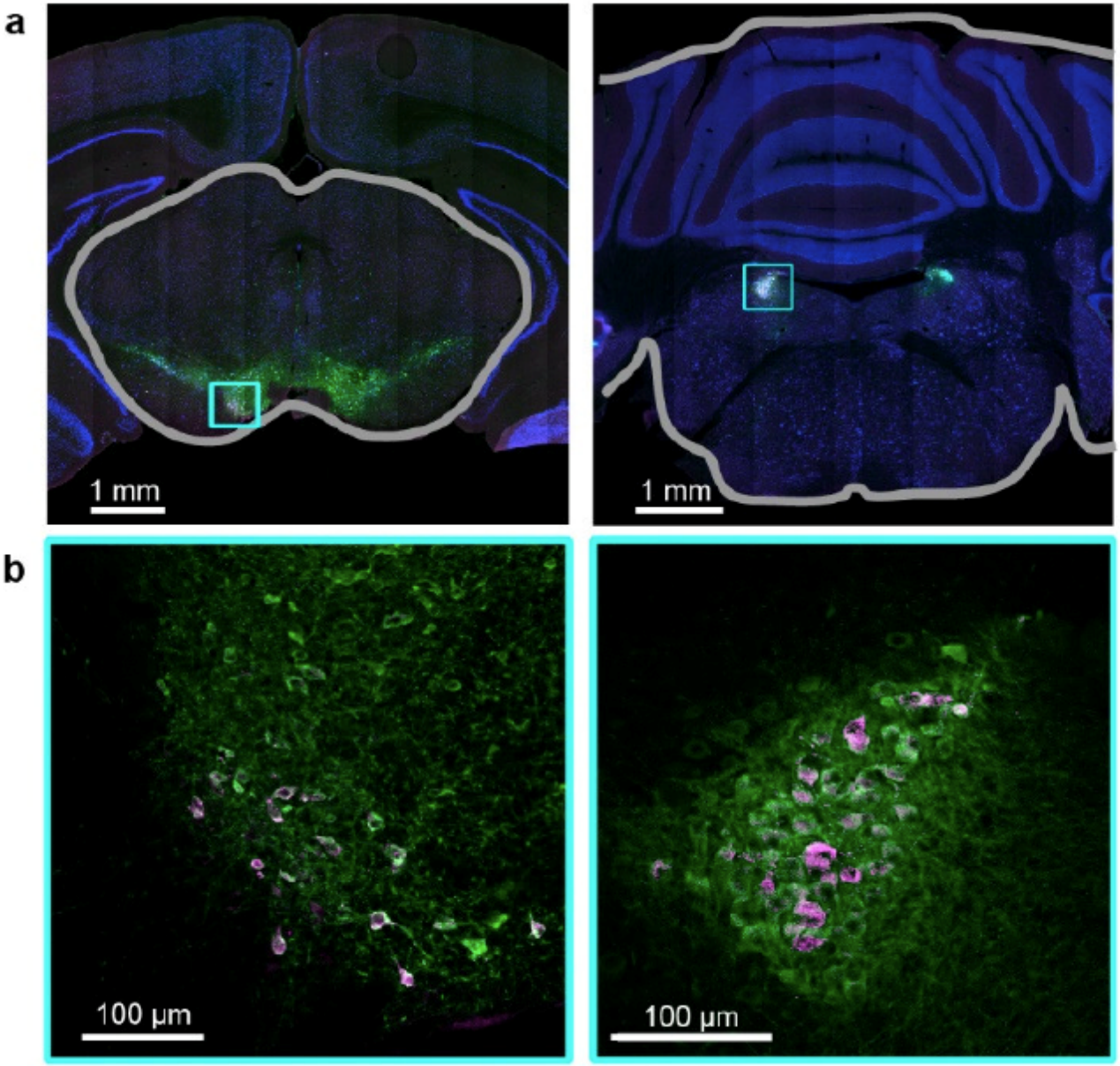
Muller *et al.*, Nature Methods, 2014

Dopaminergic and Noradrenergic projections to the cortex

Retrograde fluorescent tracer **Fluoro-Gold** Injected in the cortex

Analysis 7 days after surgery with antibody colabeling

DA neurons from substaintia nigra, NE neurons from locus coeruleus



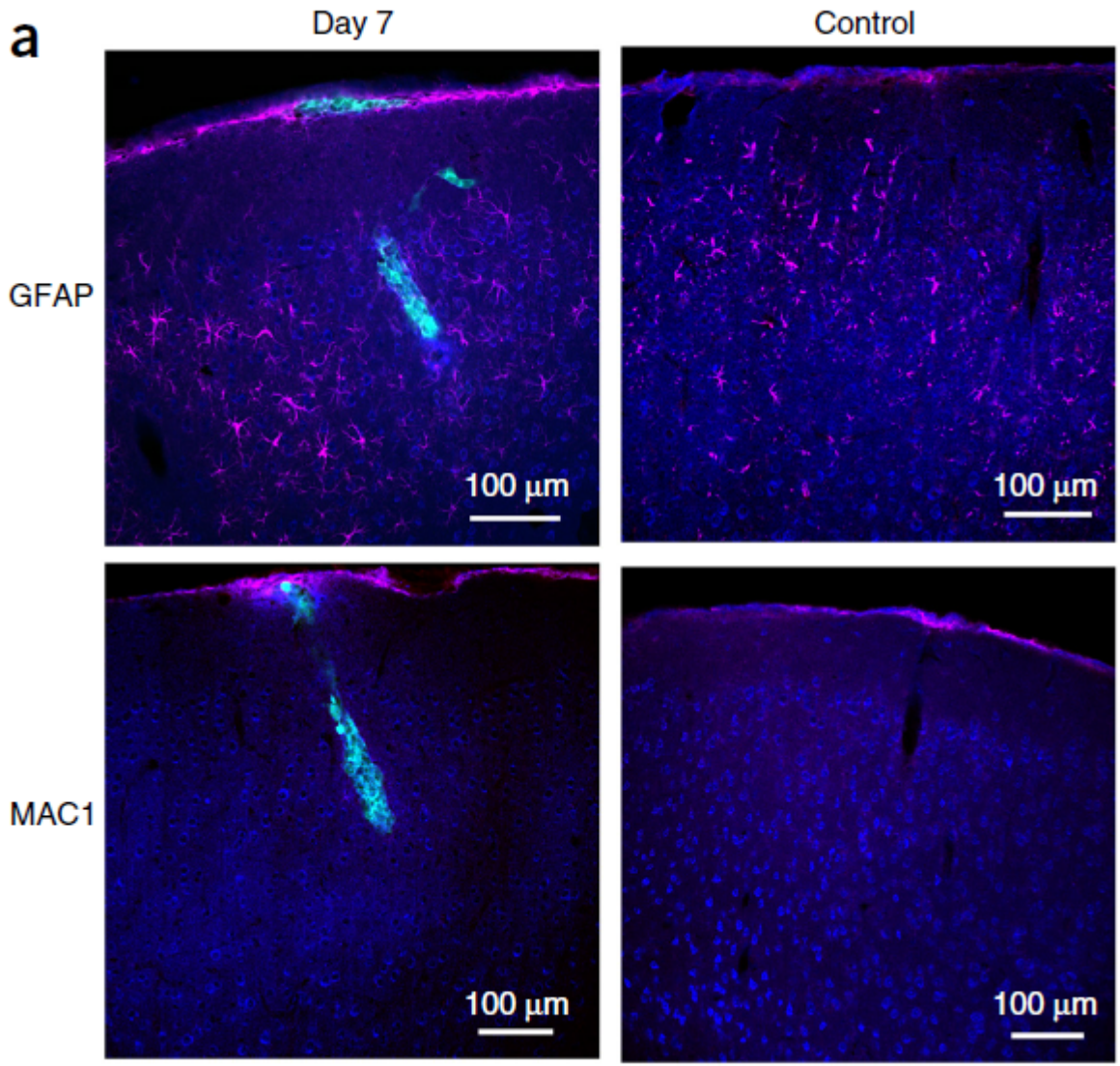
→ Electrodes implanted into either the SN or LC for electrical stimulation

In vivo characterization: cell implantation in the cortex

Stereotaxic Injection
200–300 µm below the cortical surface, i.e., layer 2/3

GFAP (magenta, astrocytes)
MAC1 (magenta, microglia)
NeuroTrace (blue)

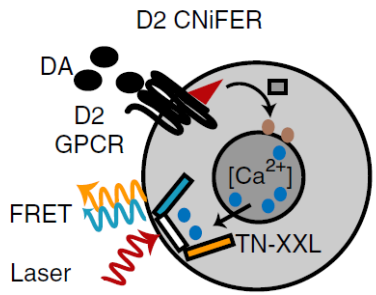
Similar presence of glial cells in both injected
and control mice
<u>No glial scars</u>
<u>Minimal damage</u>

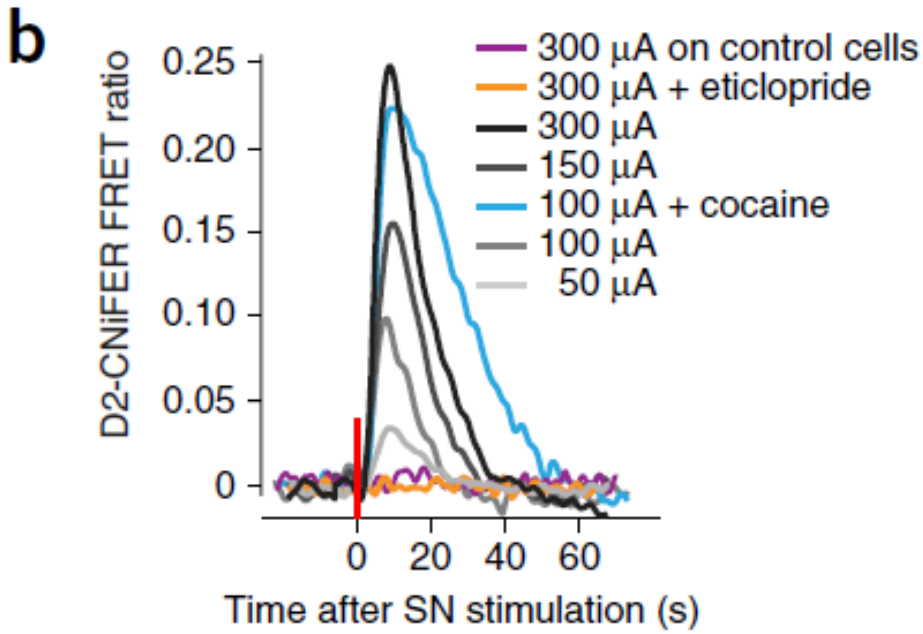


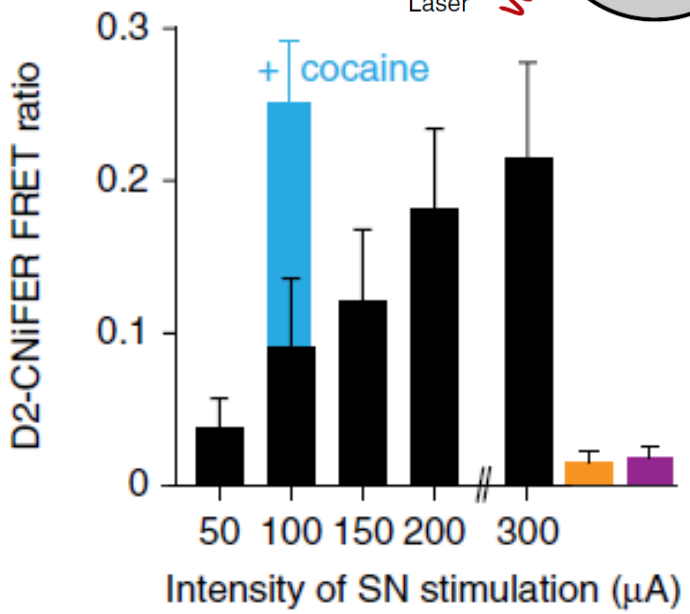
Muller *et al.*, Nature Methods, 2014

In vivo characterization: D2-CNiFERs responses

Each in vivo two-photon imaging plane contained 5–20 CNiFERs





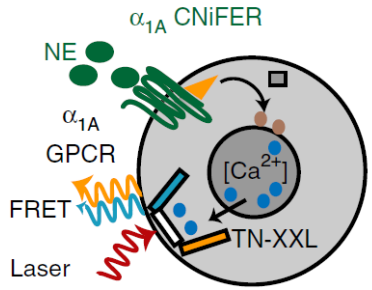


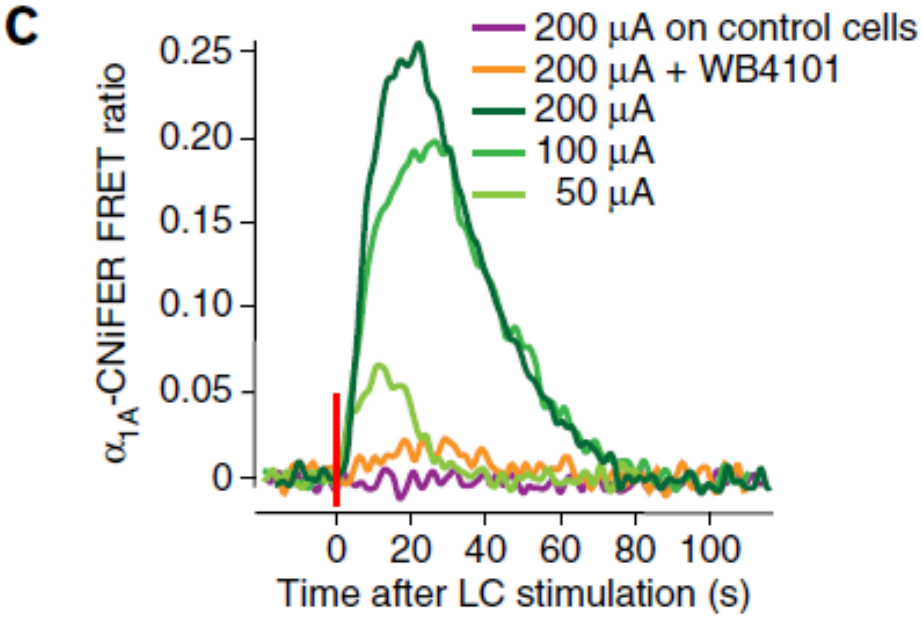
- Response within 2s of stimulation
- Amplitude of the FRET response varied with the amplitude of the stimulation
- Cocaine (15 mg mg/kg; i.p.): DA reuptake inhibitor → enhanced the size of the response
- Eticlopride (1 mg mg/kg; i.p.): D2 antagonist → suppressed the CNiFER response
- No response of control cells
- The duration of the FRET response varied from 20 s to more than 1 min

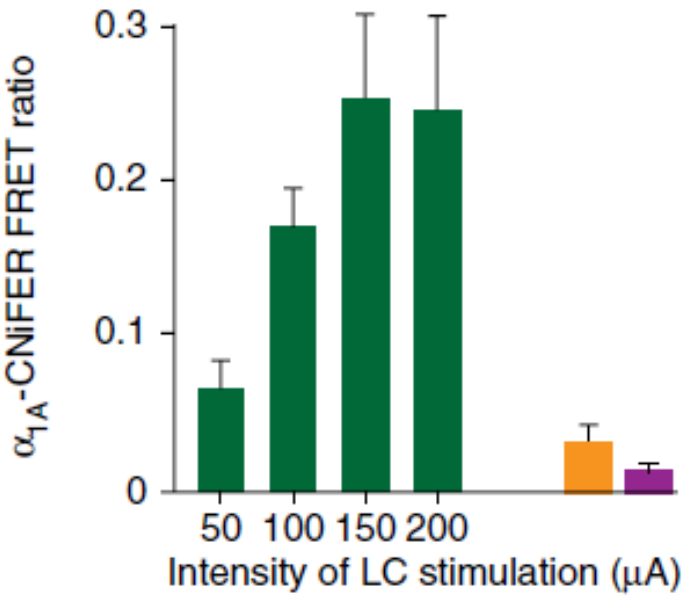
Muller *et al.*, Nature Methods, 2014

In vivo characterization: α1A-CNiFERs responses

Each in vivo two-photon imaging plane contained 5–20 CNiFERs





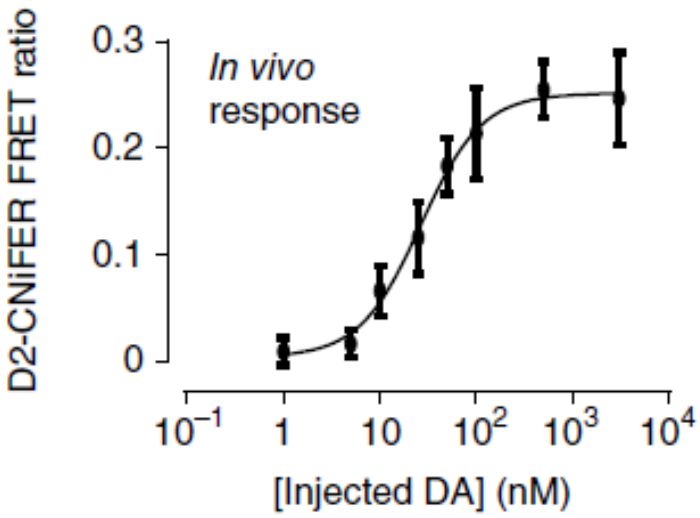


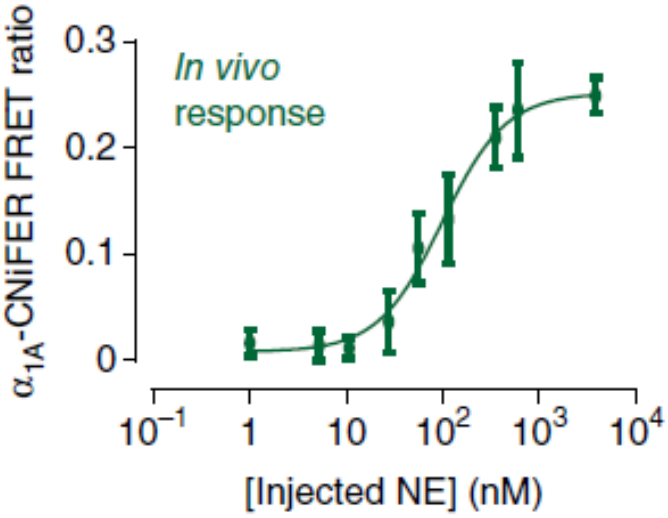
- Response within 2s of stimulation
- Amplitude of the FRET response varied with the amplitude of the stimulation
- WB4101 (2 mg mg/kg; i.p.): α1A antagonist → suppressed the CNiFER response
- No response of control cells
- The duration of the FRET response varied from 20 s to more than 1 min

Muller *et al.*, Nature Methods, 2014

In vivo characterization: dose responses

- micropipette was placed with a tip approximately 100 μm from the implant
- train of pulses of agonist delivered concurrently with a fluorescent indicator





D_2 CNiFER in vivo sensitivity to DA:
EC_{50} = 29 ± 5 nM

α1A CNiFER in vivo sensitivity to NE:
EC_{50} = 90 ± 21 nM

Can it be combined with behavioural analysis over multiple days?

Muller *et al.,* Nature Methods, 2014

Classical conditioning / Pavlovian conditioning

→ form of learning in which the ***conditioned stimulus*** (**CS**), comes to signal the occurrence of a second stimulus, the ***unconditioned stimulus*** (**US**)

Conditioning {  **CS** **+**  **US**

→ conditioned <u>cue-reward pairings increase neuronal firing in midbrain neurons</u>

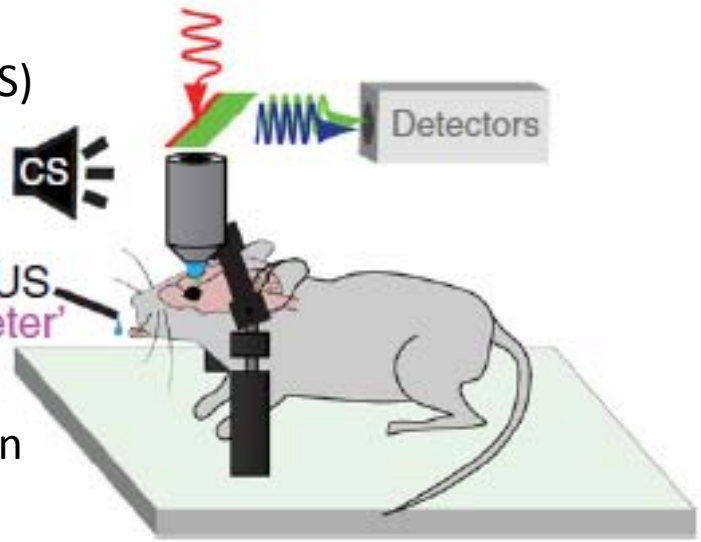
→ neurons in the LC transiently spike in response to task-relevant stimuli: possible NE increase in the cortex during conditioning ???

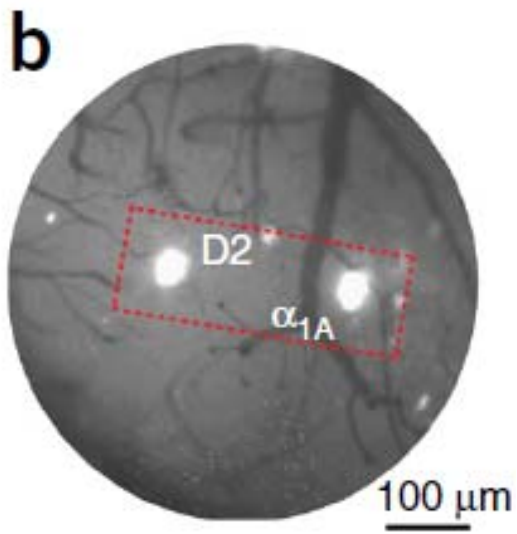
Pavlovian conditioning paradigm in head-fixed mice implanted with CNiFERs

5-s tone (CS)

3 s delay

drop of 10% sucrose solution (US)



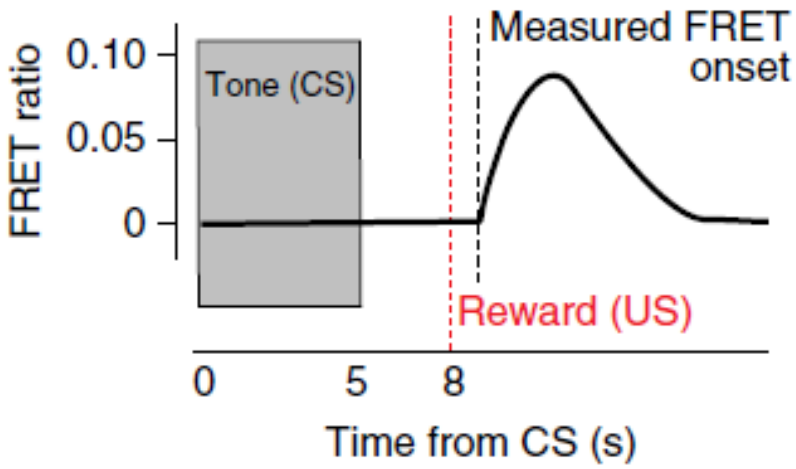


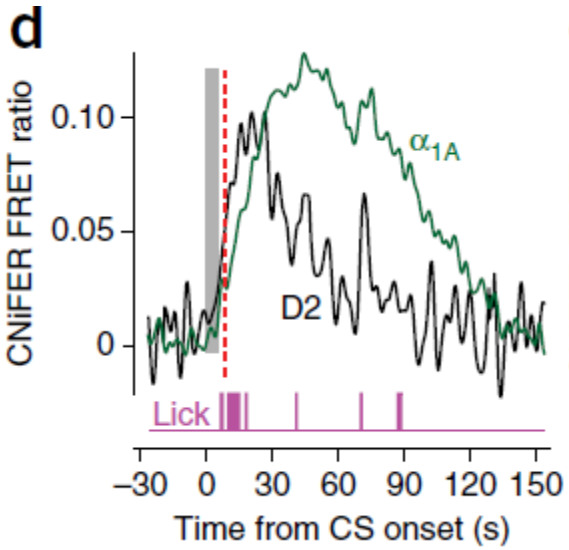
Mice injected with only D2 CNiFERs, or with D2 and **α**1A CNiFERs in discrete sites separated by ~300 **μ**m, in layers 2/3 of the frontal cortex

!!!
sucrose retrieval required the motor act of tongue protrusion and licking: another cohort implanted with M1 CNiFERs + D2 CNiFERs

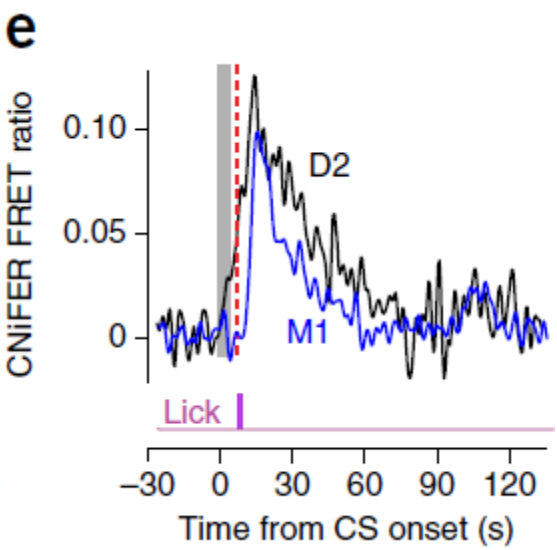
Muller *et al.,* Nature Methods, 2014

Simultaneous measurement of neuromodulators during test





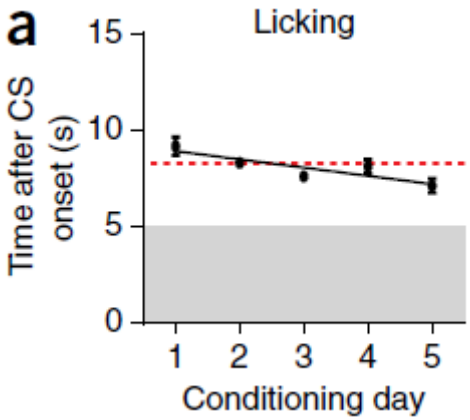
increase in the FRET ratio for both D2 and **α**1A CNiFERs



increase in the FRET ratio for both D2 and M1 CNiFERs

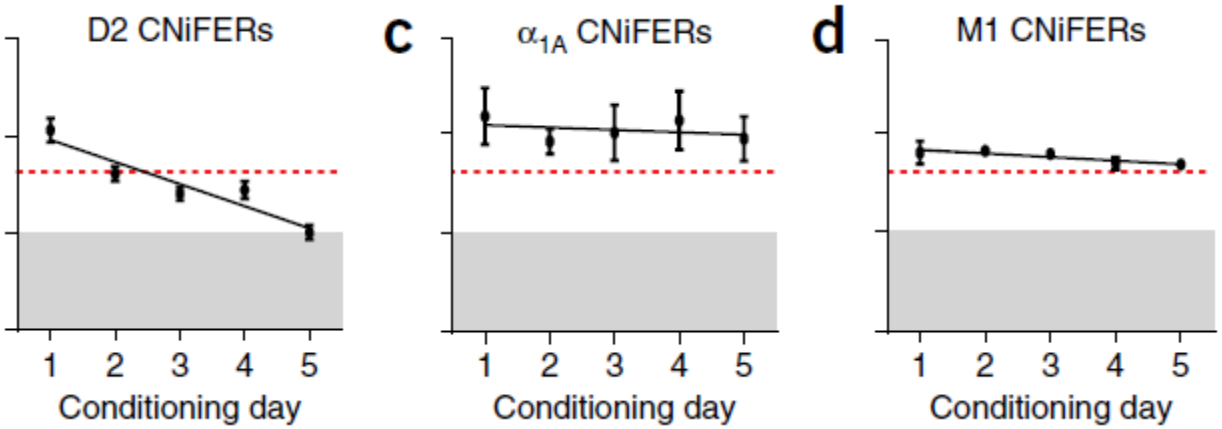
Muller *et al.,* Nature Methods, 2014

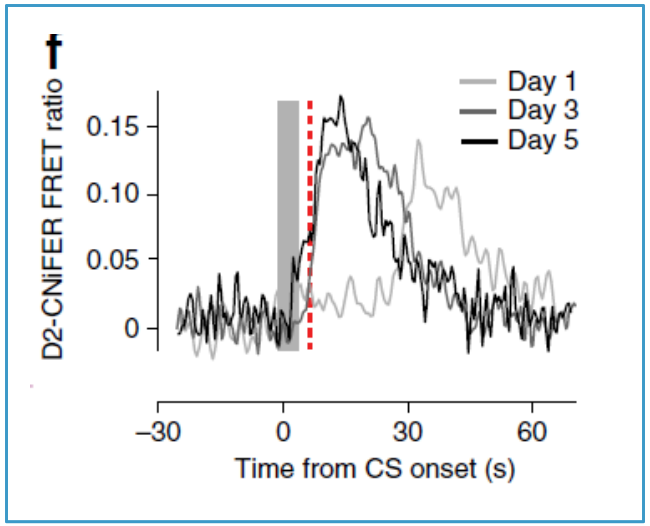
Shift with behavioural conditioning across multiple days



a statistically significant decrease in the **time to lick**, with a slope of −0.40 ± 0.14 s per day (n=13)

Does DA or NE time of release change as well?



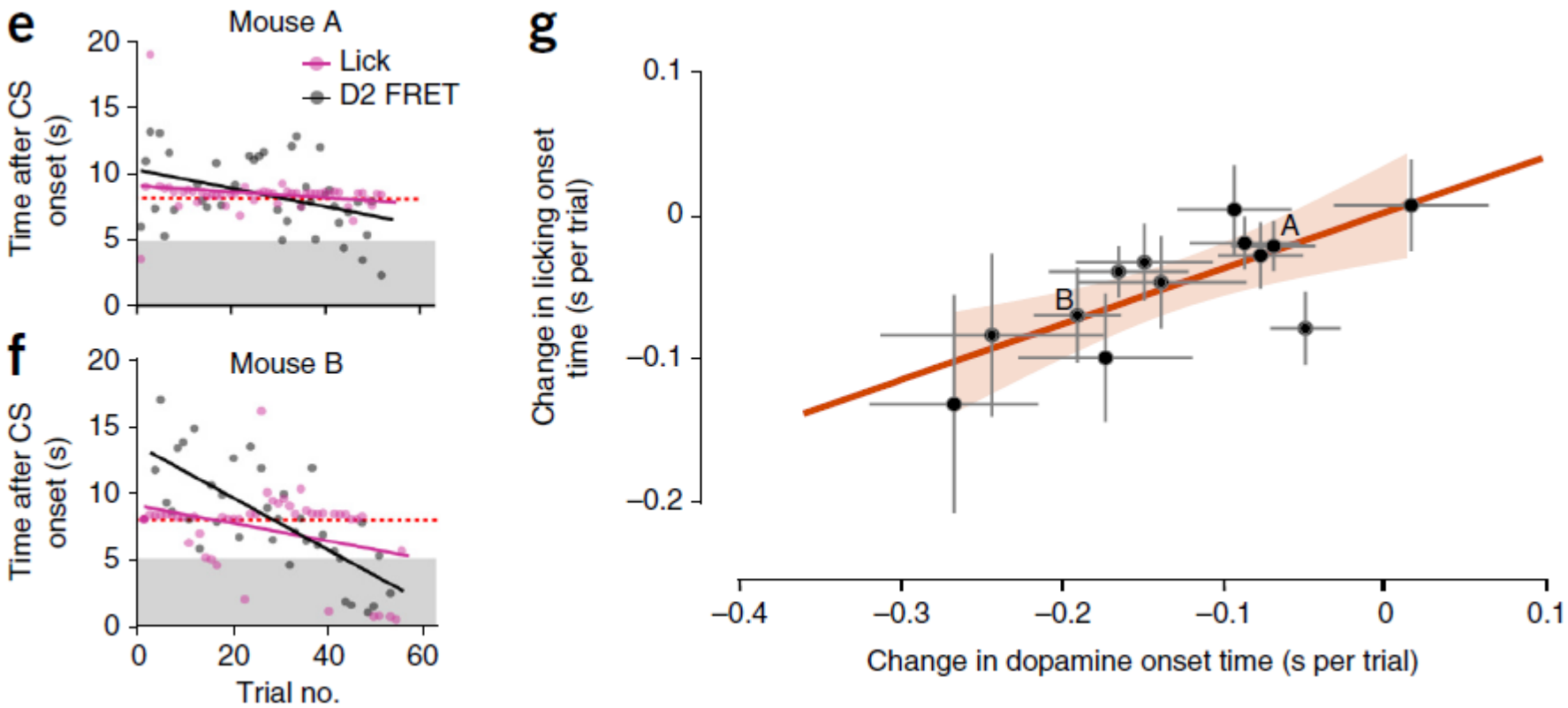


Big decrease in the onset time of the FRET ratio for dopamine release across multiple days of training: from 10.3 ± 0.6 s (13 mice) during the first day of training to 5 ± 0.3 s during the last day of training, with a slope of −1.1 ± 0.14 s per day

Muller *et al.*, Nature Methods, 2014

Correlation of DA release with anticipatory licking

→ the release of DA correlated with learning the association of CS with US



→ E: small change in the timing of DA release = no significant anticipatory licking
→ F: strong shift in DA release = significant change in anticipatory licking
→ G: strong correlation between rates of change in DA release and anticipatory licking (n=13)

→ DA release tracks the extent of learning

Muller *et al.*, Nature Methods, 2014

Conclusions

→ new family of cell-based CNiFERs for rapid, optical detection of neurotransmitters released in vivo

→ Implemented for Gi/o-coupled receptors, in addition to Gq-coupled receptors

→ Discrimination between DA and NE

→ Nanomolar sensitivity, temporal resolution of seconds

→ Little impact on the brain

→ Used for imaging experiments with head-fixed mice

Possible future improvements:

→ Different setting to study neuromodulation in freely moving animals

→ Different molecular design: to generate CNiFERs for any molecule that activates a GPCR

Muller *et al.,* Nature Methods, 2014