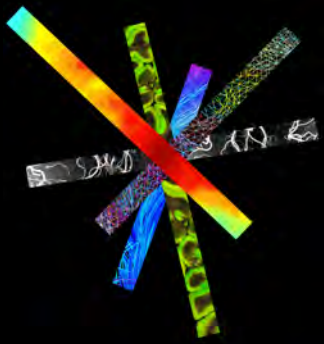




The Excitatory/Inhibitory imbalance hypothesis in autism – evidence from neuroimaging

Nouchine Hadjikhani, MD, PhD

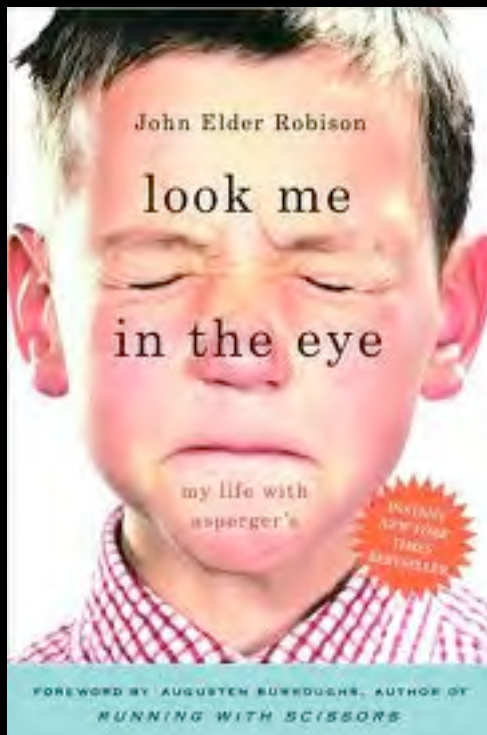
Athinoula A. Martinos Center for Biomedical Imaging

Massachusetts General Hospital

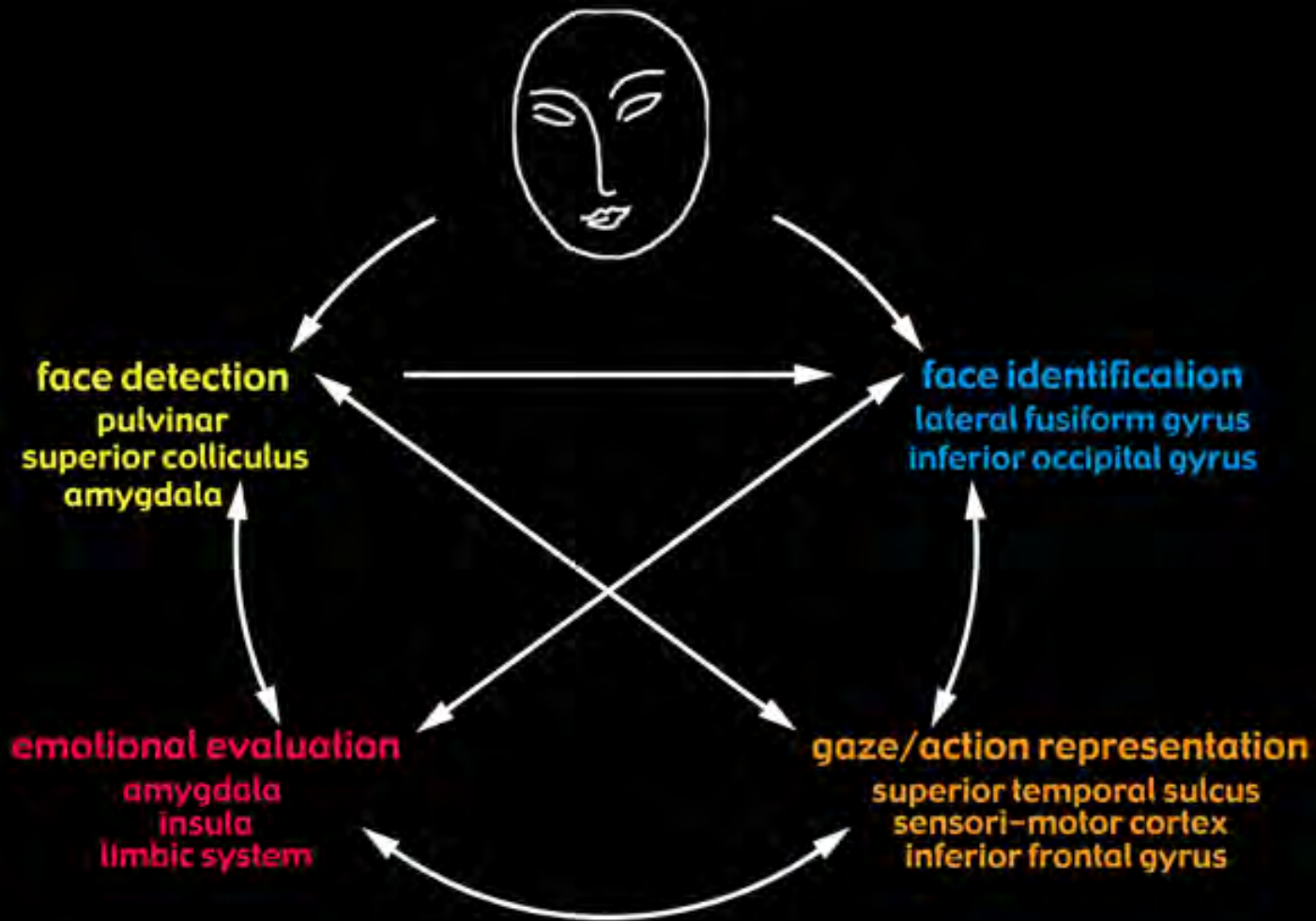
Harvard Medical School – Boston, USA

Gillberg Centrum, Gothenburg, Sweden

Looking in the eyes

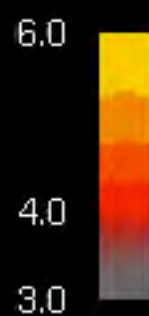


Network of face processing areas

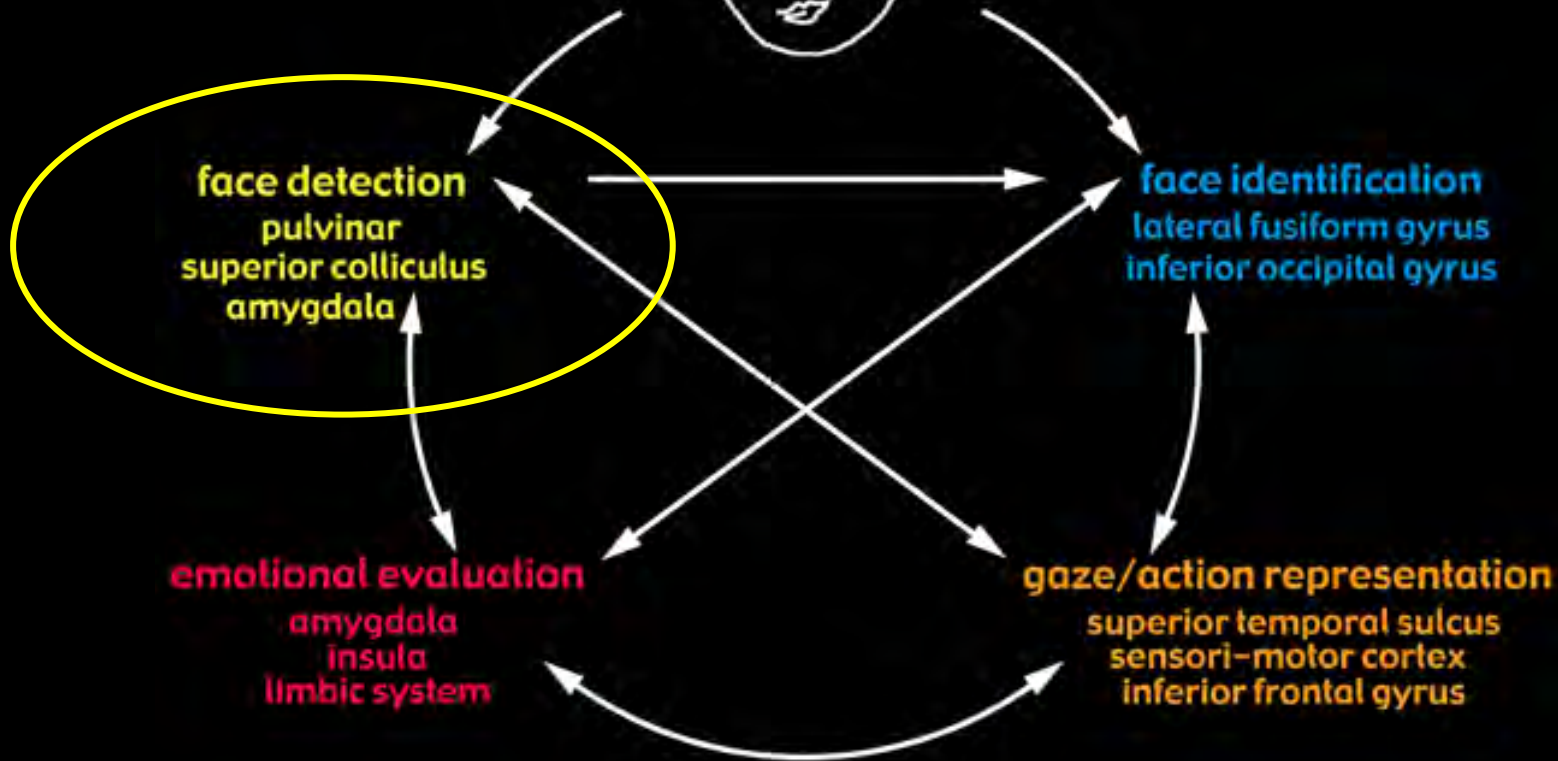




$t = 0.0$ ms
dSPM SNR = 3.0
3.0 .. 4.0 .. 6.0



Network of face processing areas



Newborn are naturally and instinctively attracted by faces

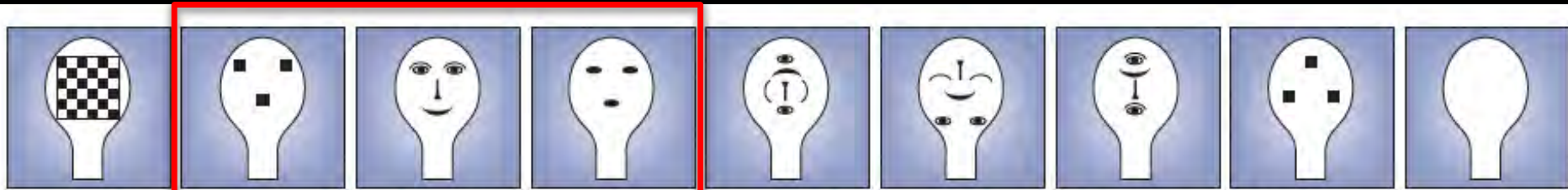


Figure 2 | Both schematic and realistic stimuli have been used to test newborns' preferences for face-related stimuli.

Third trimester **fetuses** are instinctively attracted by faces!

Please cite this article in press as: Reid et al., The Human Fetus Preferentially Engages with Face-like Visual Stimuli, *Current Biology* (2017), <http://dx.doi.org/10.1016/j.cub.2017.05.044>

Current Biology
Report

CellPress

The Human Fetus Preferentially Engages with Face-like Visual Stimuli

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²Department of Physics, Lancaster University, Lancaster LA1 4YB, UK

³Department of Obstetrics and Gynaecology, Blackpool NHS Trust, Blackpool FY3 8NR, UK

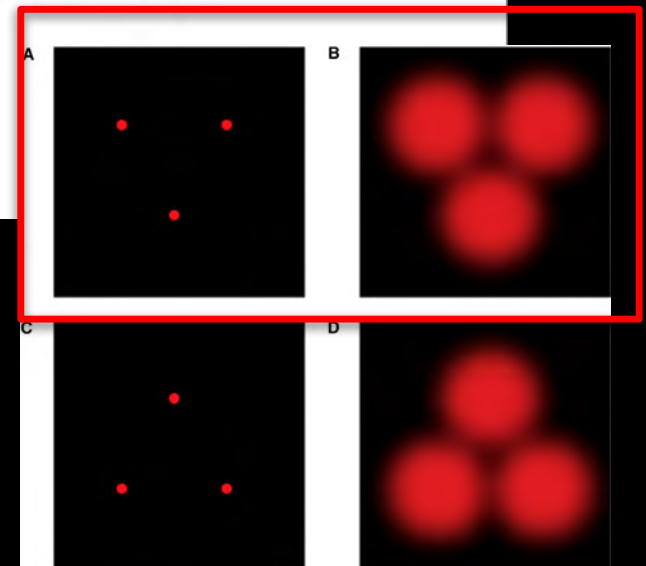
⁴Medical and Sports Sciences, University of Cumbria, Lancaster LA1 3JD, UK

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<http://dx.doi.org/10.1016/j.cub.2017.05.044>



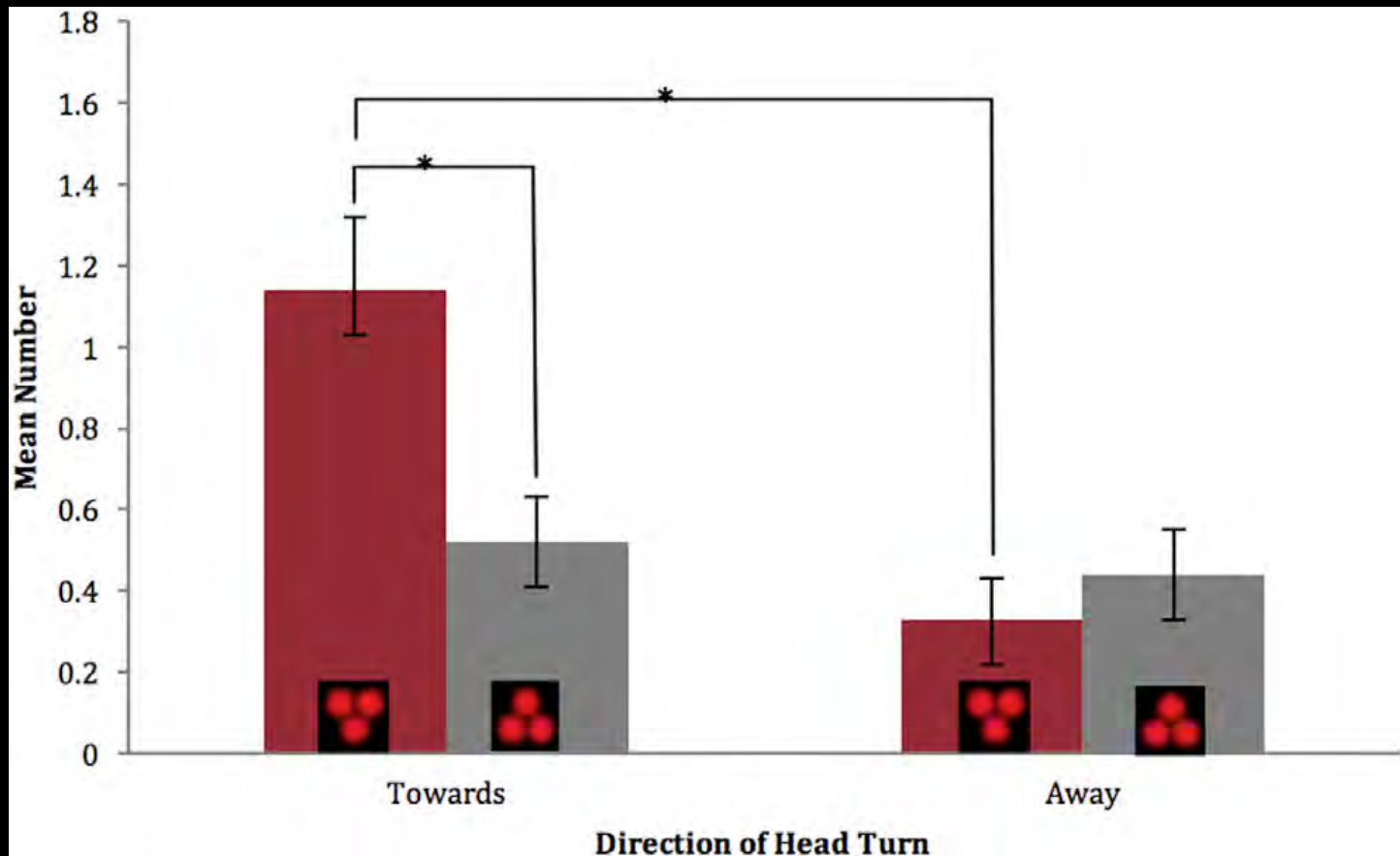


Figure 2. The Mean Number of Fetal Head Turns to the Stimuli

The mean number of head turns made toward (left two bars) and away (right two bars) for face-like (red) and non-face-like (gray) stimuli. Error bars represent standard errors. Stars indicate significant differences between conditions, with the brackets representing the relevant comparisons.

Stimulus that is optimal to elicit face-related attention in newborns

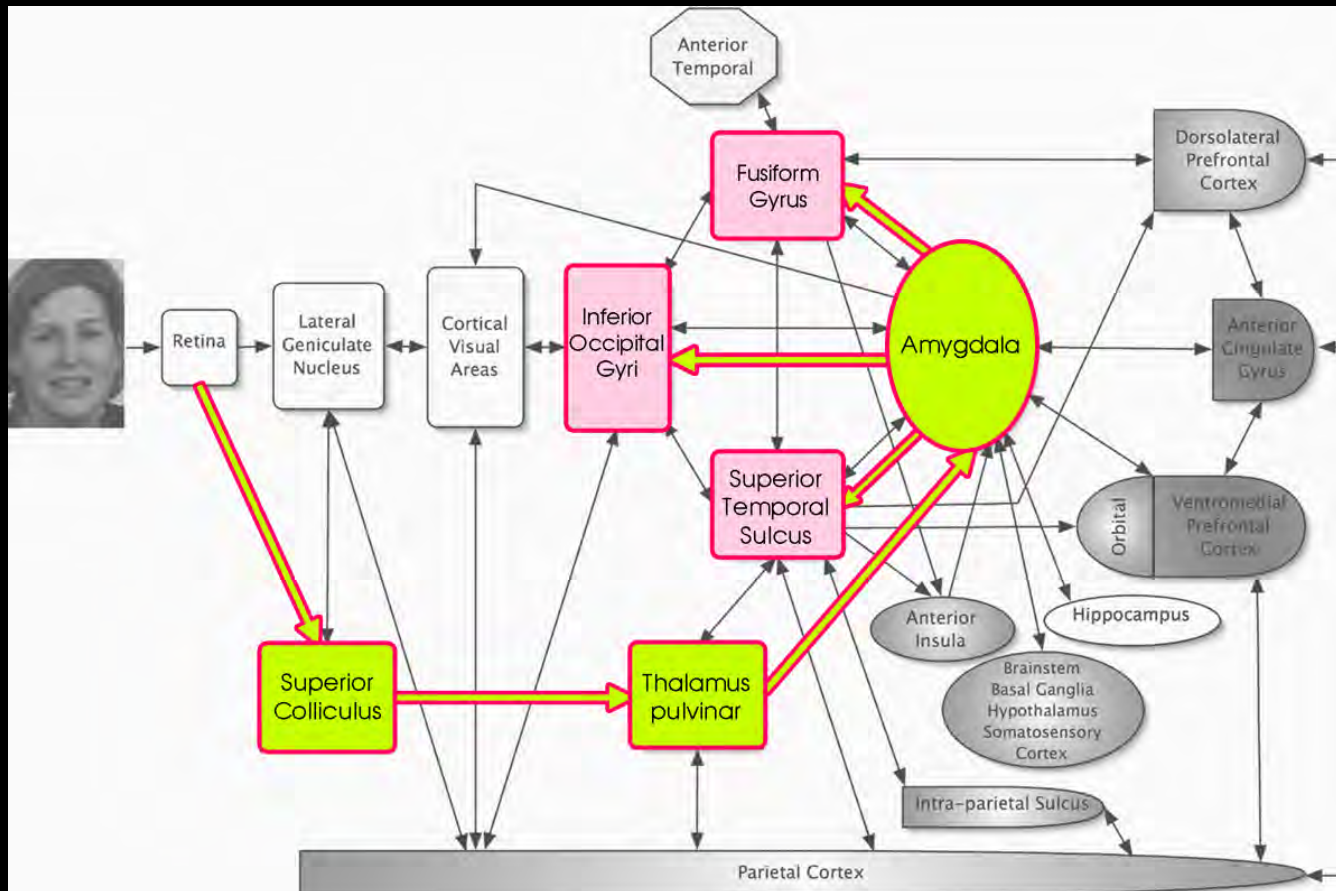


Newborns have complex face representation!



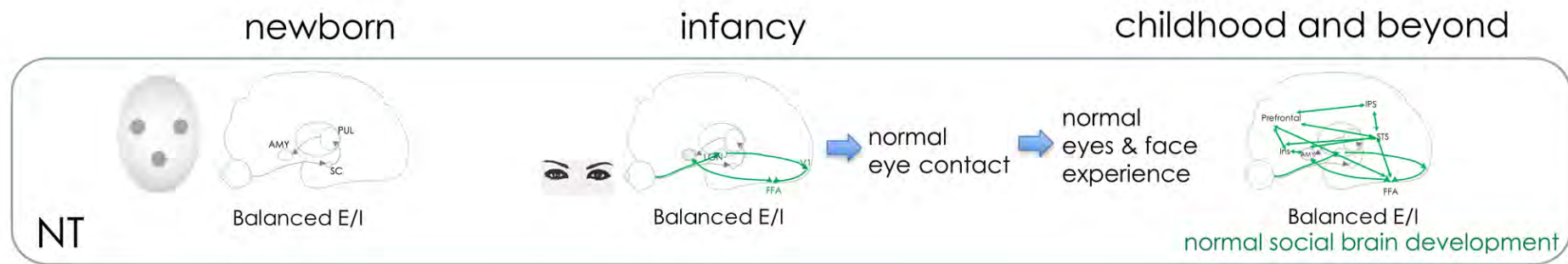
- In the newborn, it is the SUBCORTICAL system that supports face perception
- Fast pathway
- Enables rapid face detection
- Face detection activates other cortical regions that become important in the adult social brain
- The subcortical route is engaged by eye-contact (both in infants and adults)
- It remains during adult life, and is the basis for blindsight

The subcortical route



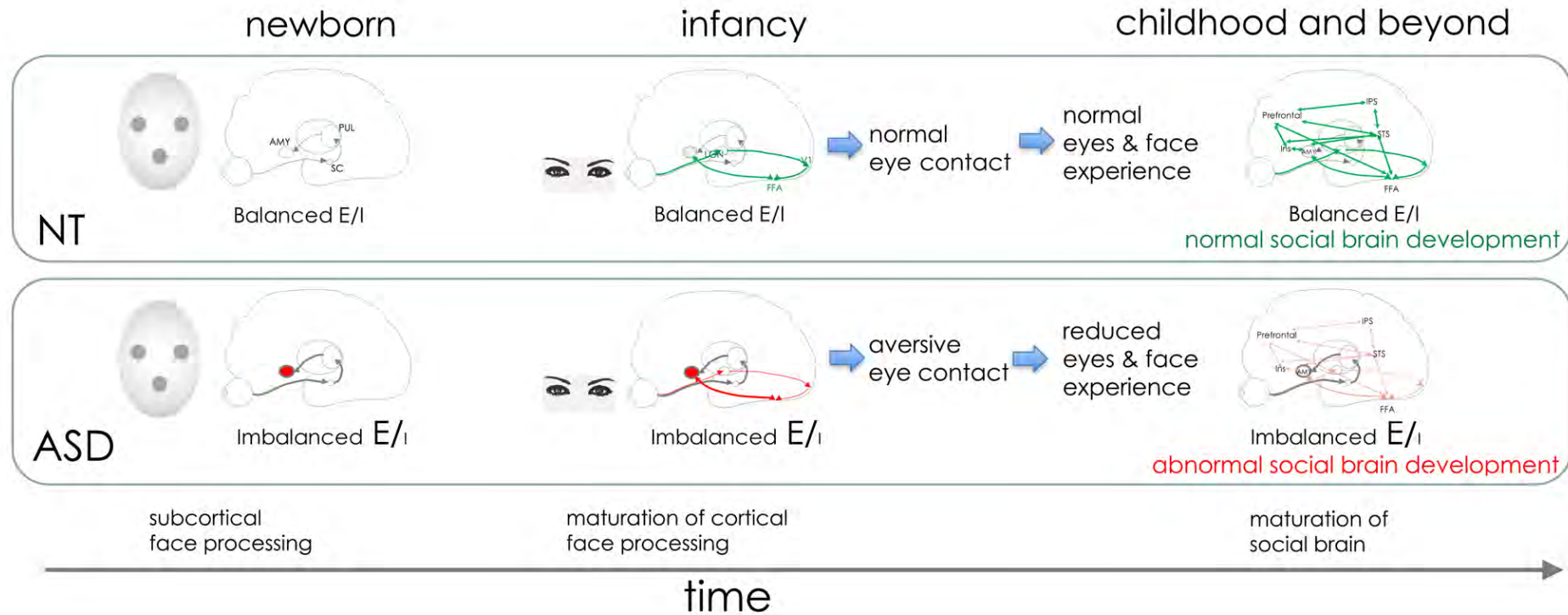
The developmental E/I imbalance hypothesis

Normal development



- First hours onwards: the subcortical route gets activated by general configuration of faces
- Overtime, the subcortical route, in particular the amygdala, becomes sensitive to eyes, but not oversensitive
- Normal eye-contact leads to normal eyes, gaze and face experience and normal social brain development

The developmental E/I imbalance hypothesis

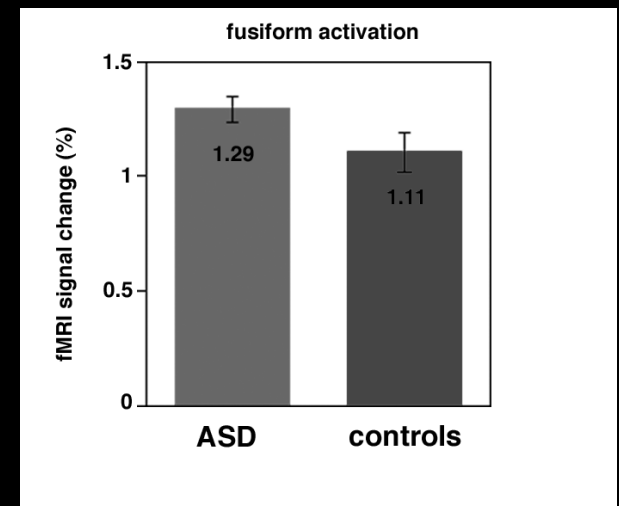
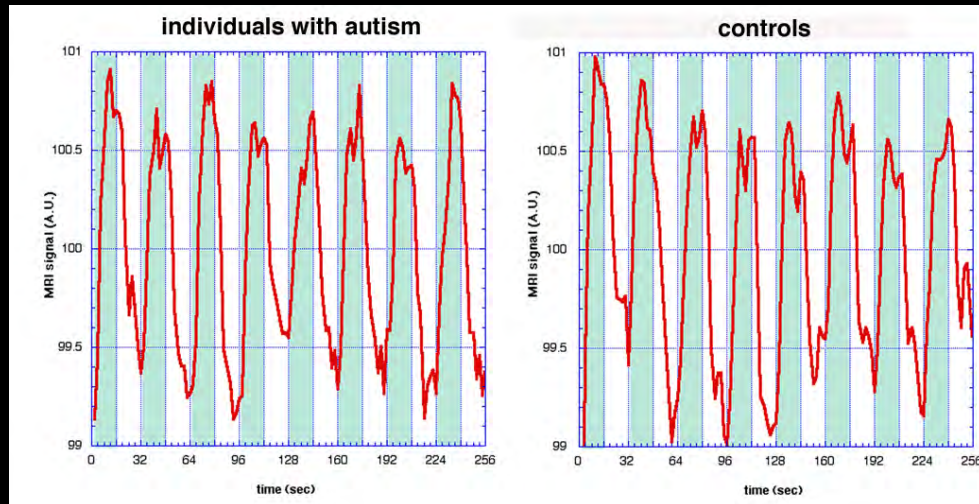
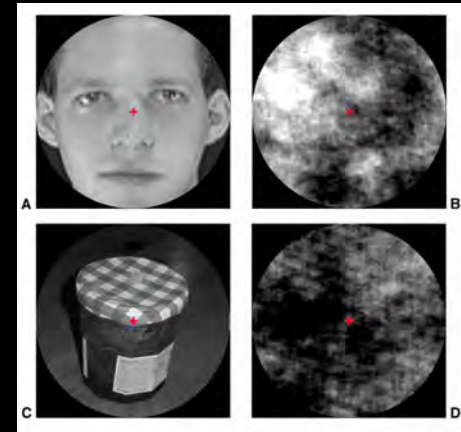
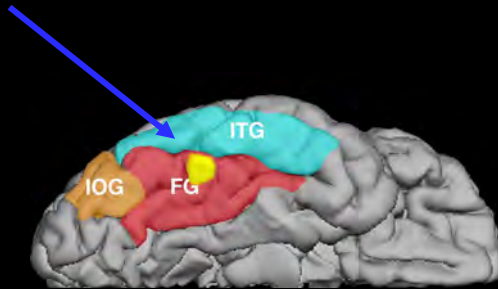


E/I imbalance:

- First hours onwards: the **subcortical**, in particular the **amygdala** route gets **overly activated** by general configuration of faces
- The subcortical route becomes **over-connected** (hebbian rule)
- Later, the amygdala becomes **over sensitive to eyes**
- **Eye contact** is **aversive**, is **avoided**, leading to **reduced eyes and face experience**, and ultimately to **abnormal brain development**

Constraining to look in the eyes for neutral faces: fMRI signal change in the FFA

FFA



Activation of the FFA depends on where they look!

Gaze fixation and the neural circuitry of face processing in autism

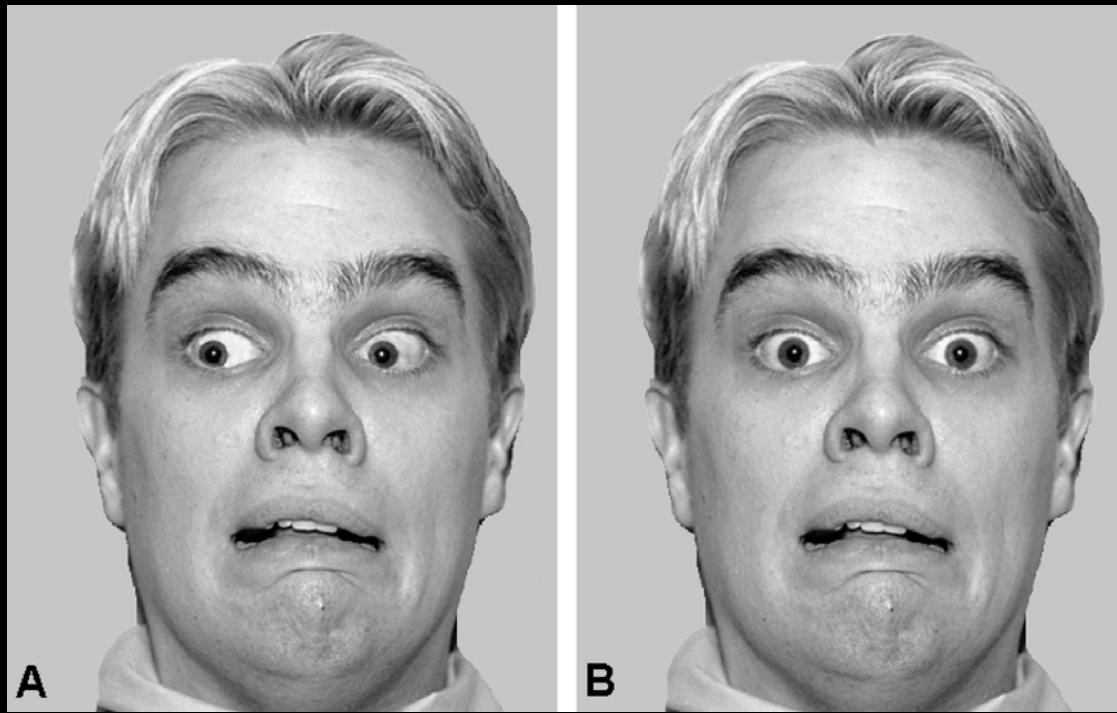
Kim M Dalton^{1,2}, Brendon M Nacewicz², Tom Johnstone², Hillary S Schaefer², Morton Ann Gernsbacher^{1,3}, H H Goldsmith^{1,3}, Andrew L Alexander^{1,2,4} & Richard J Davidson¹⁻⁴

Diminished gaze fixation is one of the core features of autism and has been proposed to be associated with abnormalities in the neural circuitry of affect. We tested this hypothesis in two separate studies using eye tracking while measuring functional brain activity during facial discrimination tasks in individuals with autism and in typically developing individuals. Activation in the fusiform gyrus and amygdala was strongly and positively correlated with the time spent fixating the eyes in the autistic group in both studies, suggesting that diminished gaze fixation may account for the fusiform hypoactivation to faces commonly reported in autism. In addition, variation in eye fixation within autistic individuals was strongly and positively associated with amygdala activation across both studies, suggesting a heightened emotional response associated with gaze fixation in autism.

Evidence for an
abnormality of the
subcortical pathway

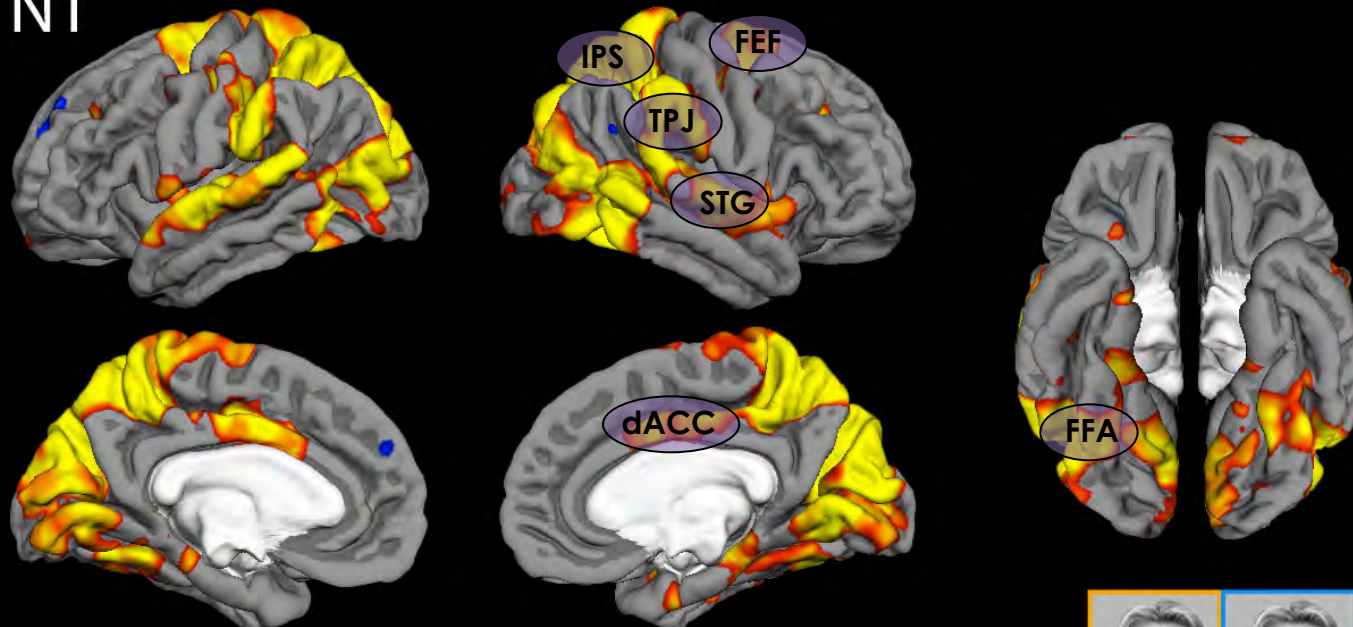
"Pointing with the eyes – brain imaging of joint attention deficits in ASD"



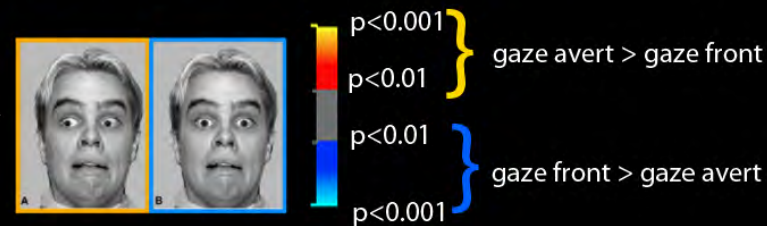


- Making 'theory of mind' inferences by integrating social cues in faces is essential
- In a fearful face, averted gaze signals the presence of a danger, whereas direct gaze is more ambiguous
- How do participants with ASD perceive fearful faces gazing towards an unseen danger?

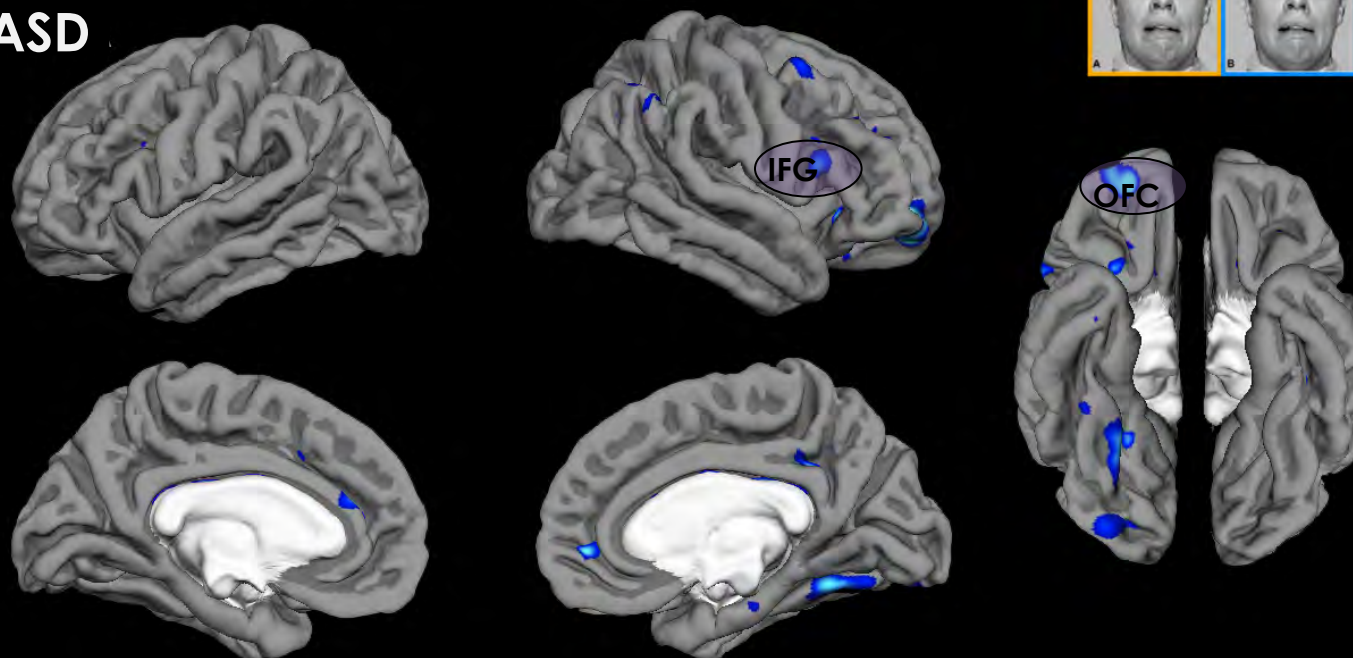
NT



n=22;
19 males, 23.7±5.9
years



ASD

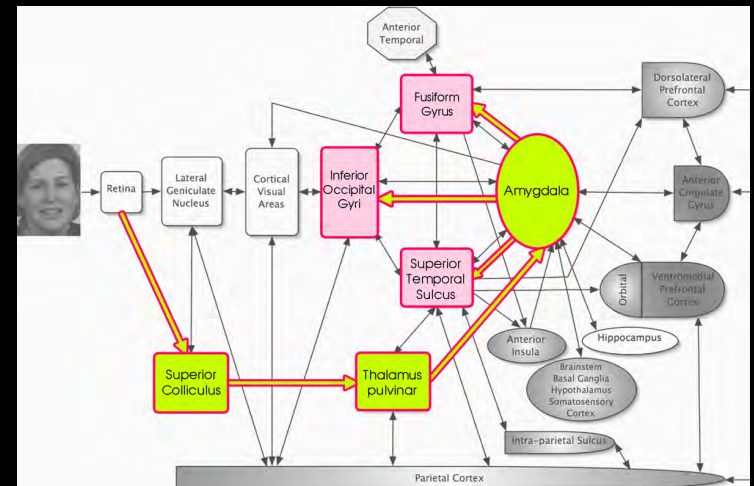
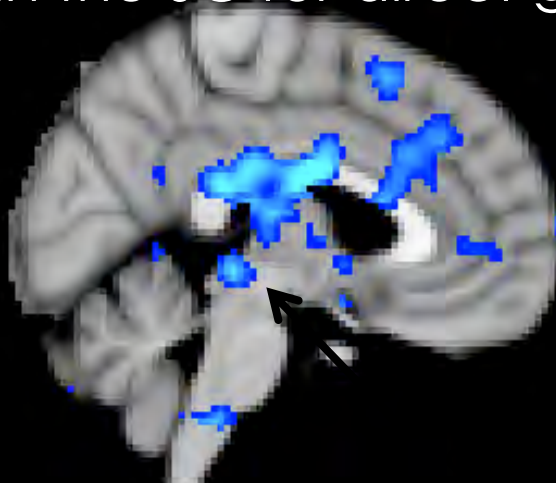


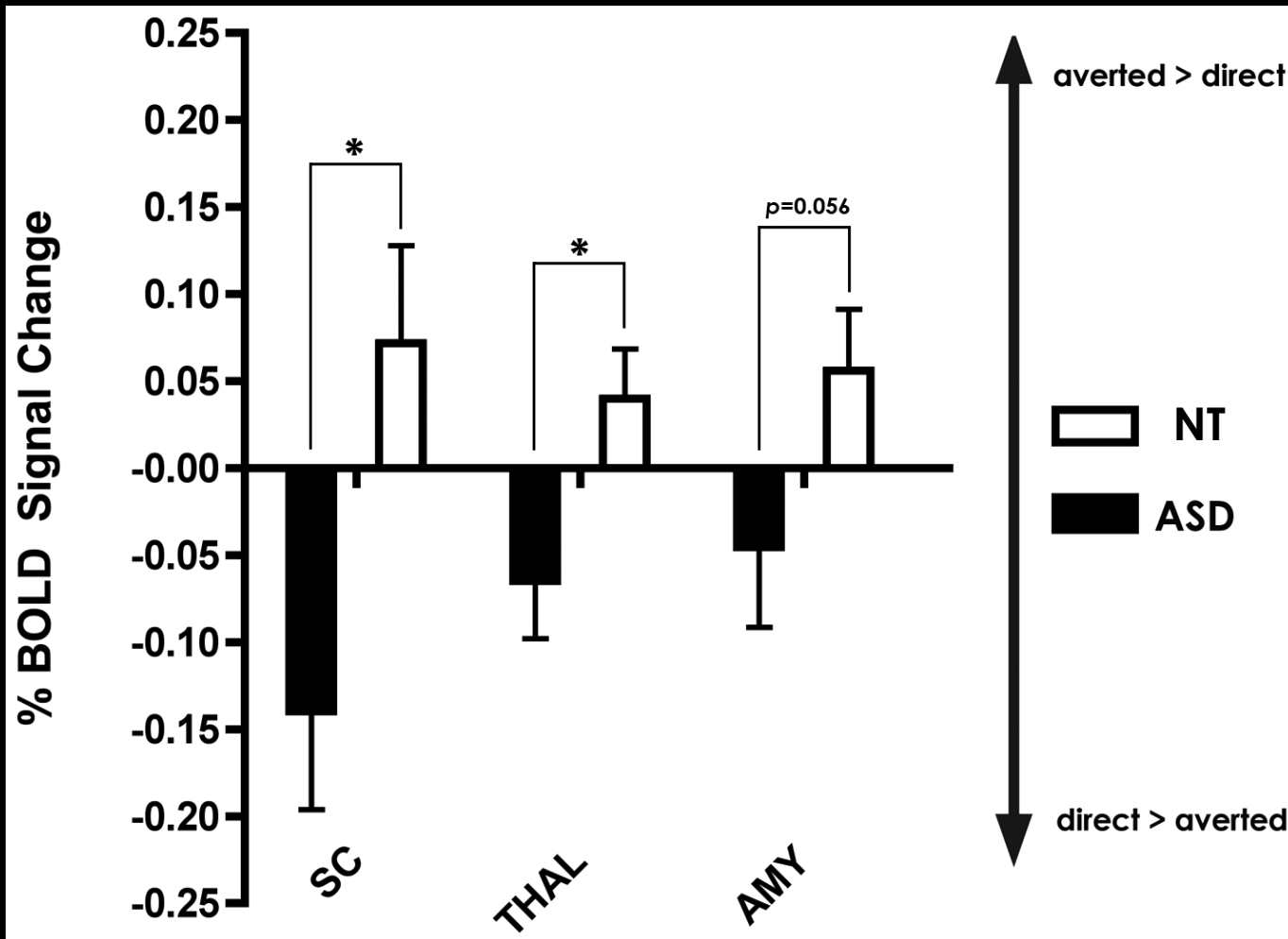
n=22;
19 males, 27.6±7.7
years

NT: More activation for averted gaze in fearful face



ASD: Almost similar activation for both conditions – more activation in the SC for direct gaze





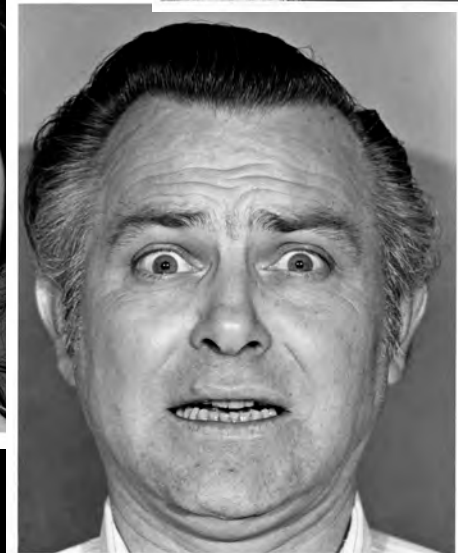
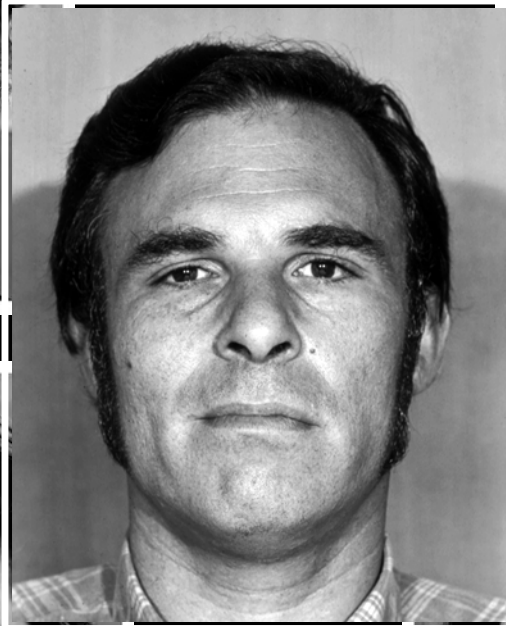
Hypersensitivity to low intensity fearful faces in autism when fixation is constrained to the eyes



Constraining to look in the eyes for emotional faces

- Previous studies examining brain activation in ASD for emotional faces did not control that participants looked in the eyes
- They all used very exaggerated expressions

Ekman emotional faces



happy

angry

fear

40 % intensity



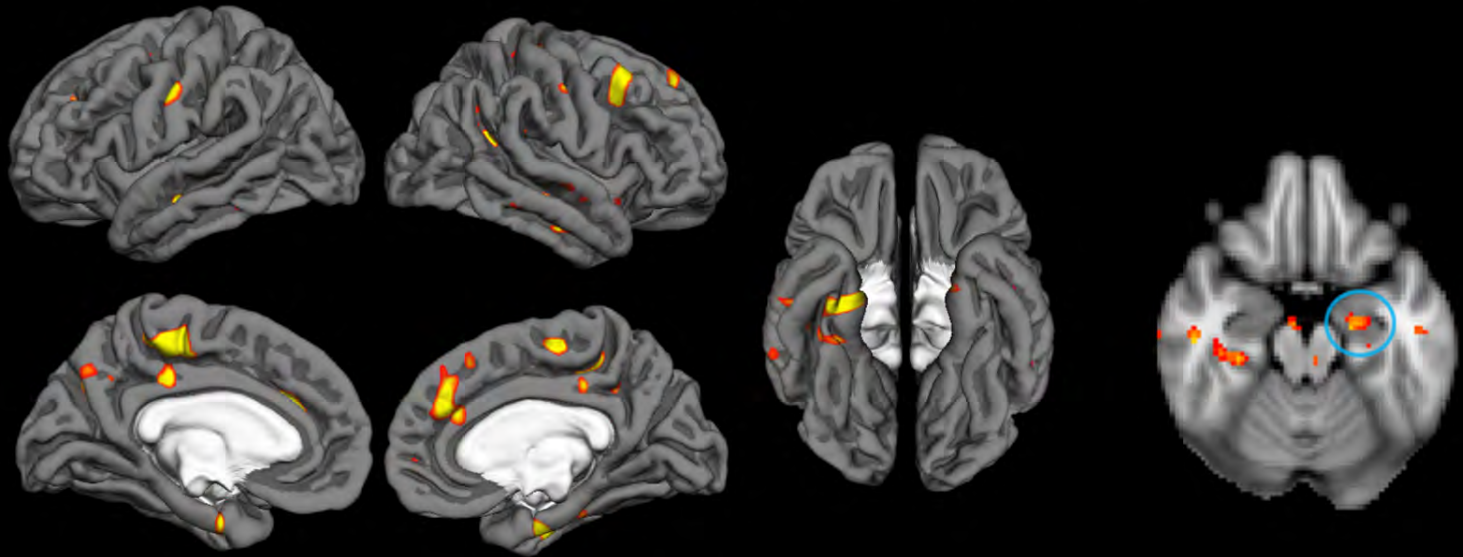
100 % intensity



Experimental design

- ASD: $n=27$; 23.6 ± 9.9 years
- NT: $n=21$; 19.7 ± 7.7 years
- Stimuli presented in blocks, pseudo-random order
- 16 blocks, with 8 different identities in each
- Happy 40%, Happy 100%, Angry 40%, Angry 100%, Fear 40%, Fear 100% and Neutral
- Each stimulus shown for 300ms, followed by 1200 ms red fixation cross
- In $\frac{1}{2}$ of the blocks, red fixation cross turned blue in one trial, and participants had to press a button (to control for attention)

ASD have **more** activation than NT for
low intensity fear



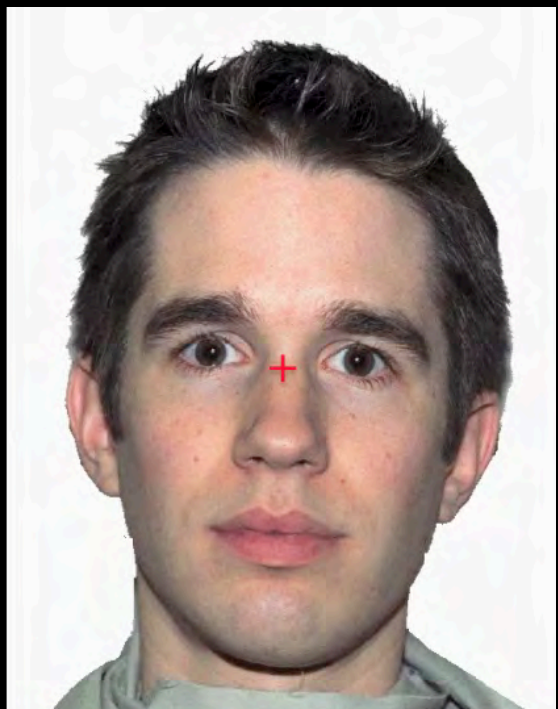
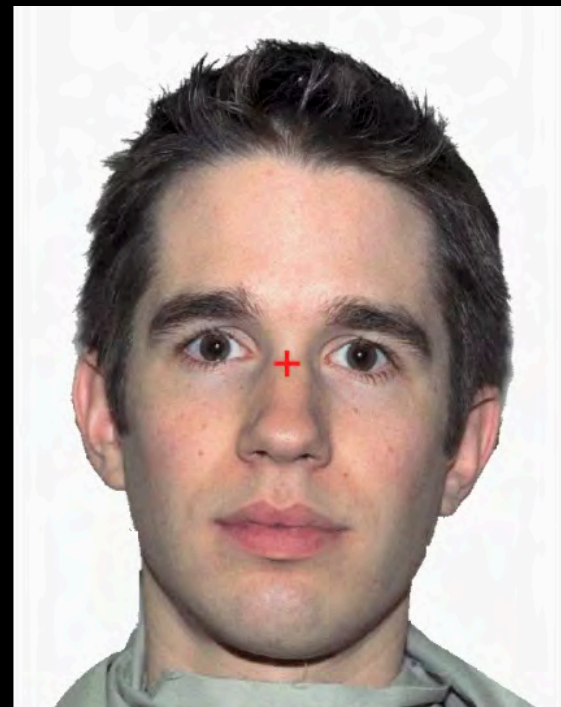
ASD > NT

Lassalle et al.,
HBM 2017

Hyperactivation of the subcortical system in ASD when constrained to look at the eyes

Experimental design

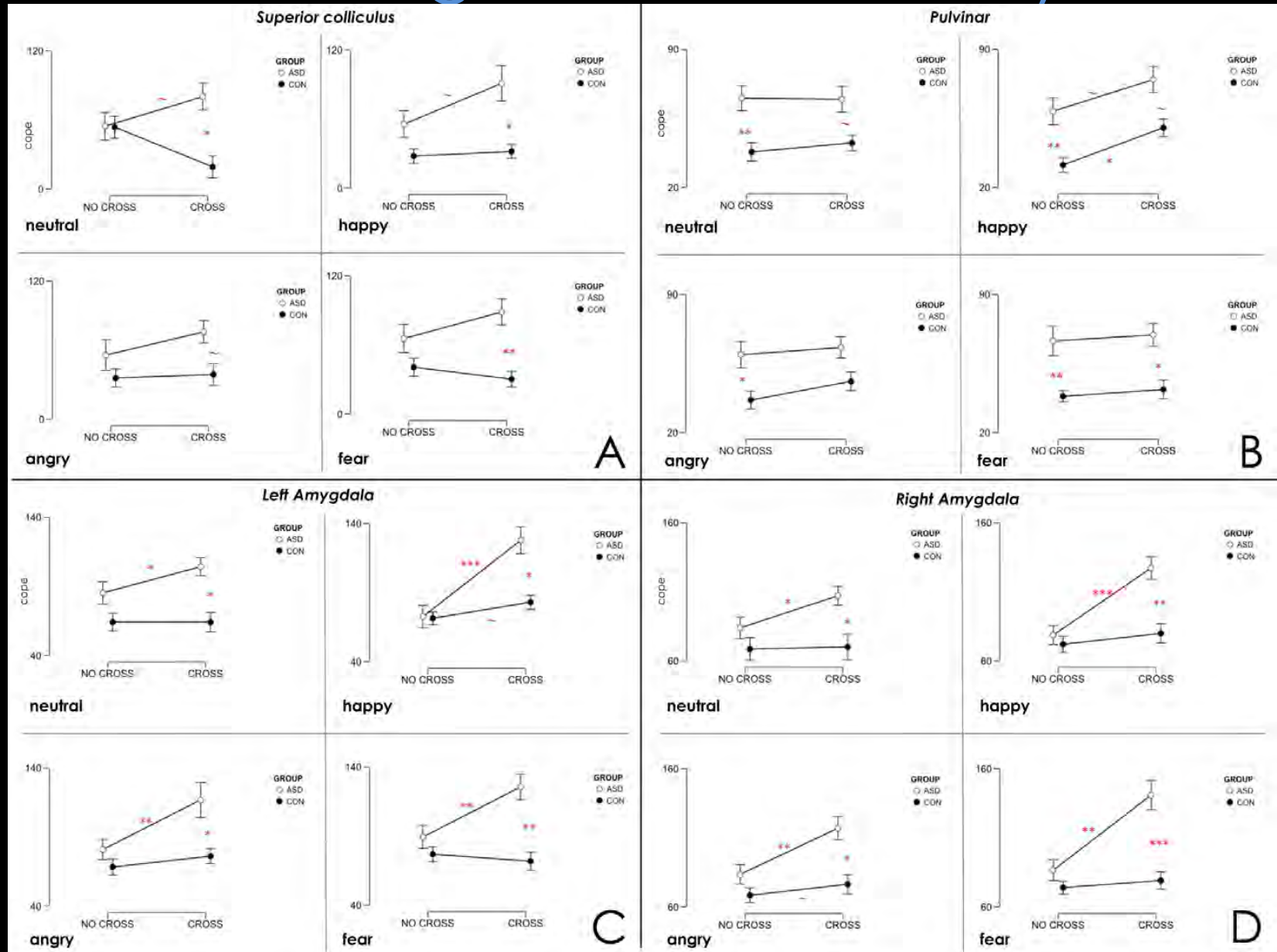
- ASD: $n=23$; 22.6 ± 1.8 years
- NT: $n=20$; 23.3 ± 1.8 years
- 24 movies created from NimStim database (Happy, Angry, Fear and Neutral)
- Each stimulus lasts 5 seconds: 3 dynamic + 2 static with final expression
- Red fixation cross between movies for 1 second – press if blue
- One version with central cross (CROSS), one without (NO CROSS)
- 2 runs, half participants saw NO CROSS first



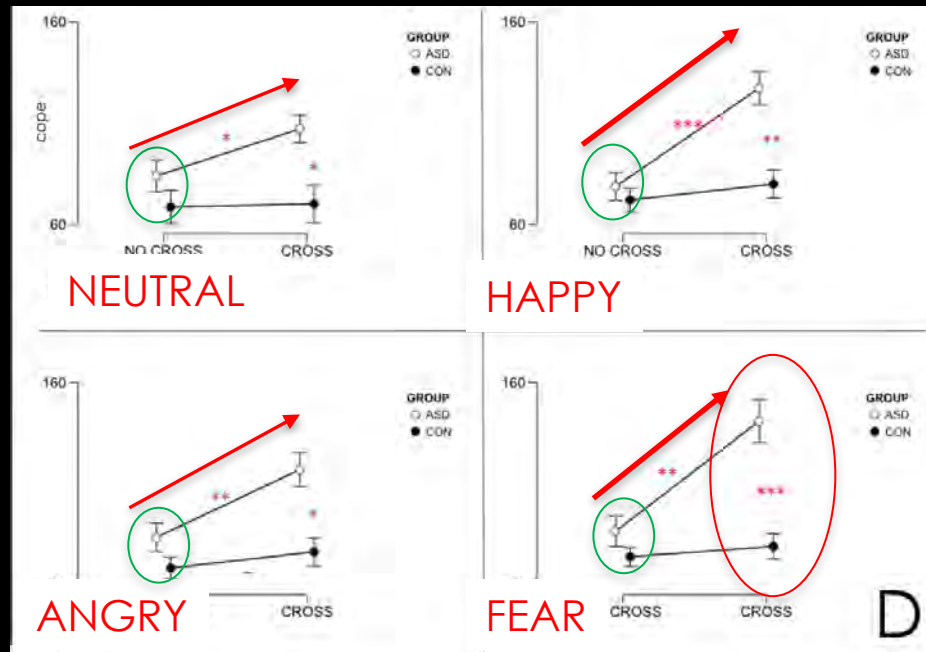
Experimental design

- For each participant, first compare CROSS vs NO CROSS condition
- Then average of CROSS vs. NO CROSS in ASD and in NT
- Compare groups in regions of interest: subcortical system
 - SUPERIOR COLLICULUS
 - THALAMUS PULVINAR
 - AMYGDALAS

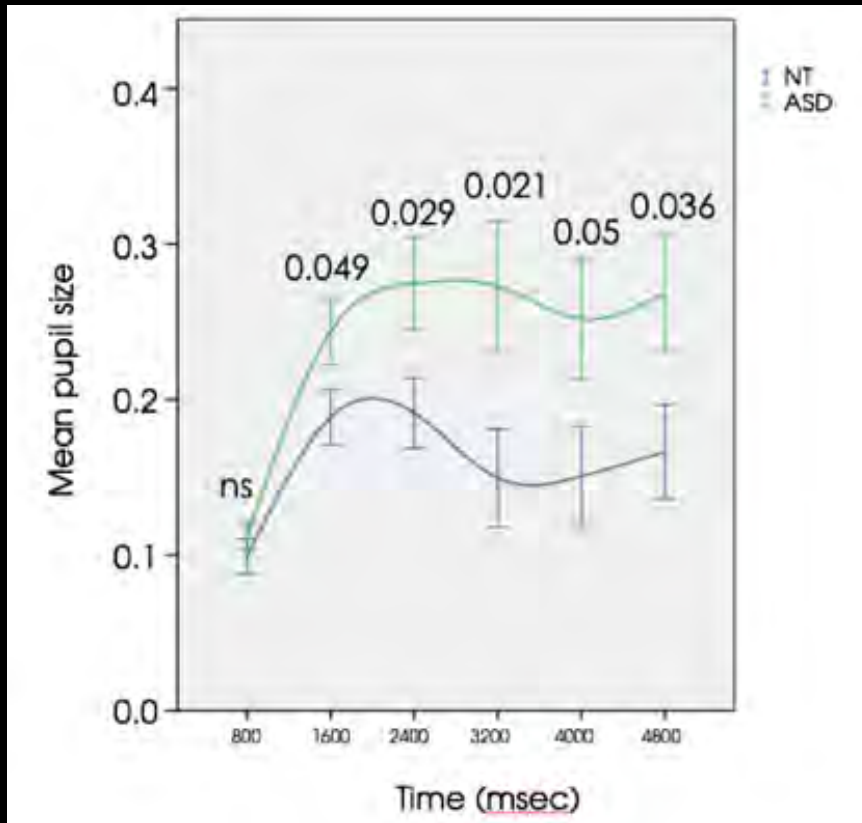
Constraining to look in the eyes



Amygdala



Data from eye-tracking



Constraining to look at eyes results in greater pupil dilation in ASD, meaning greater arousal

Conclusions

- Aberrant activation in the subcortical pathway in ASD when constrained to look in the eyes
- Eye contact is experienced as stressful
- Even for positive emotions

ASD individuals are oversensitive to eye contact, and their avoidance is to reduce over arousal!

Improving emotional face perception
in ASD with a diuretic (bumetamide):
behavioral and
fMRI data

Why a diuretic ?

- Evidence for neuronal excitability dysfunction in ASD – high prevalence of epilepsy (~30%) and abnormal EEG in about 60%
- Anecdotal reports of paradoxical effect of GABA agonist Valium on ASD
- GABA – excitatory role during pregnancy, but becomes inhibitory at birth

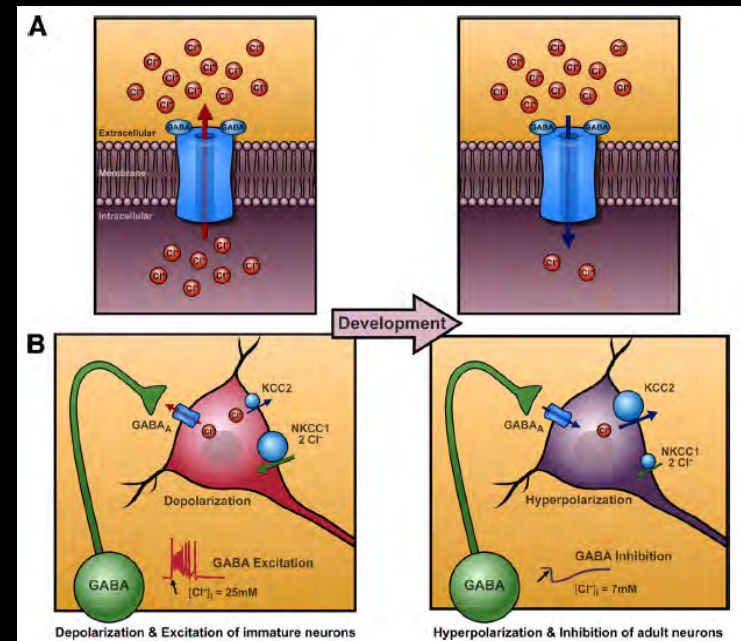
Why a diuretic ?

Switch from excitatory to inhibitory linked to decrease in the amount of intracellular chloride

Switch also linked to oxytocin

Q: Are neurons in ASD reacting as immature neurons?

What will happen if we 'force them to behave as mature' by removing intracellular chloride, with a diuretic, bumetanide?



Improving autism symptomatology with a bumetanide

- 2010: pilot open-label study on 5 patients, behavioral improvement with very little side effects (Lemonnier & Ben-Ari, Acta Paediatrica 2010)
- 2012: double blind cross over study on 60 participants: significant improvement in autistic symptomatology (Lemonnier et al., Translat Psy 2012)
- 2013: proof of concept pilot behavioral and imaging study on 9 (7+2) adolescents before and after 10 months of treatment (Hadjikhani et al, Autism 2014)
- 2017: Multicenter phase 2B in children and adolescents: 88 ASD participants (2-18 year old) (Lemonnier et al, Translational Psychiatry)
- 2018: Second proof of concept study with brain imaging, showing reduced eye-contact aversion (Hadjikhani et al, Scientific Reports)

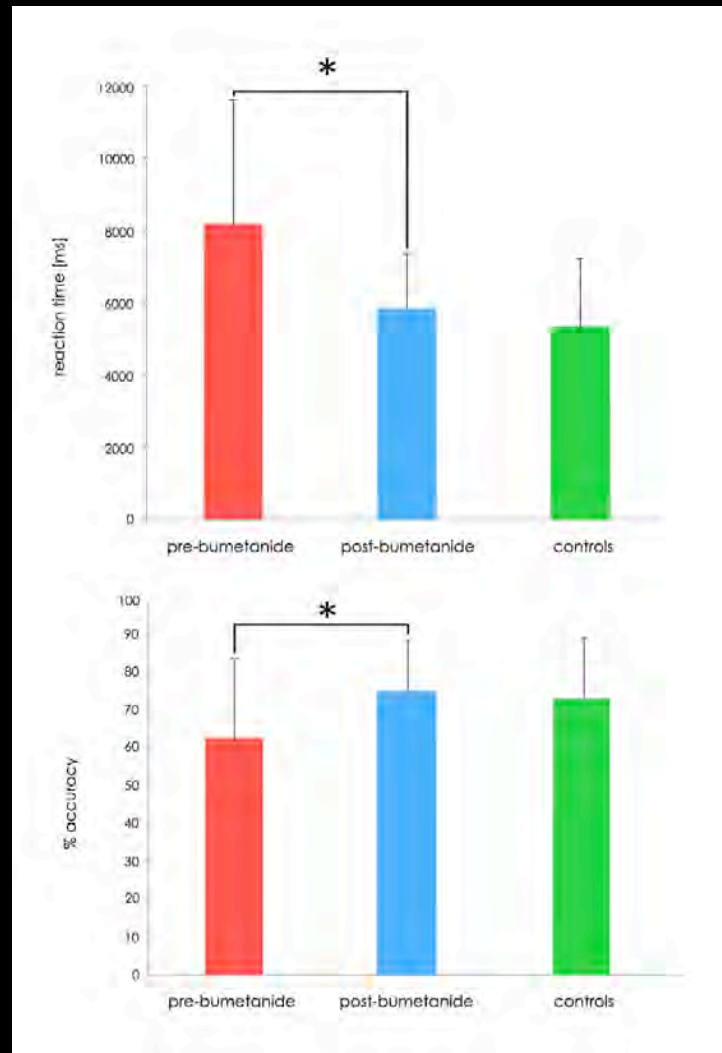
Proof of concept pilot studies

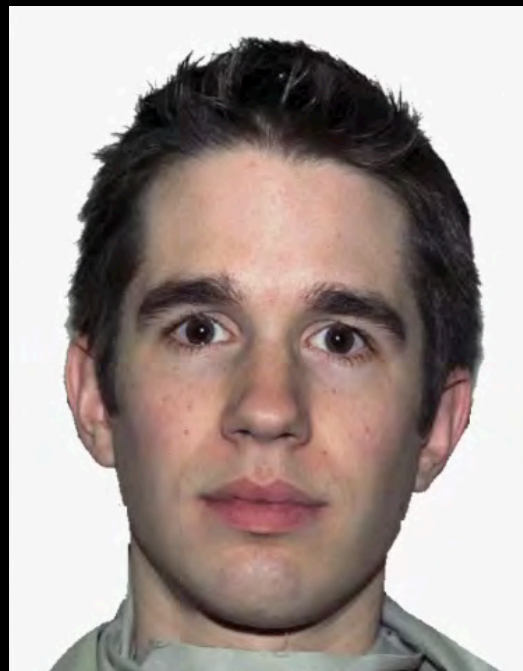
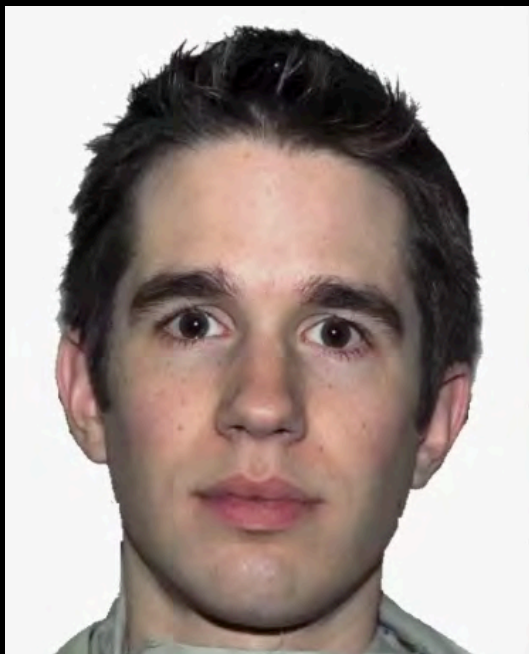
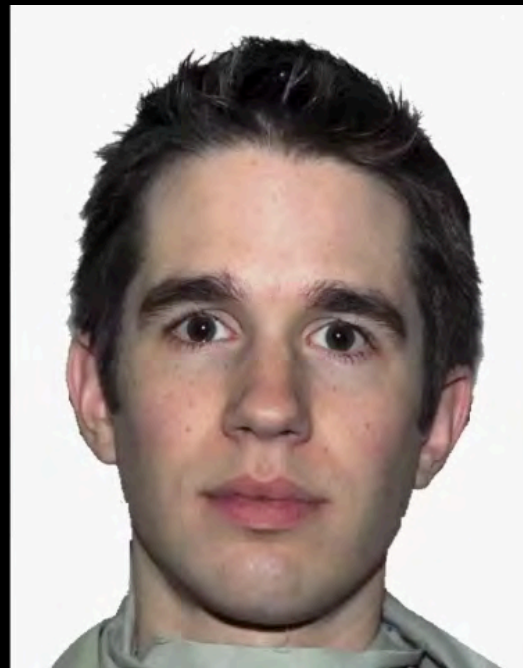
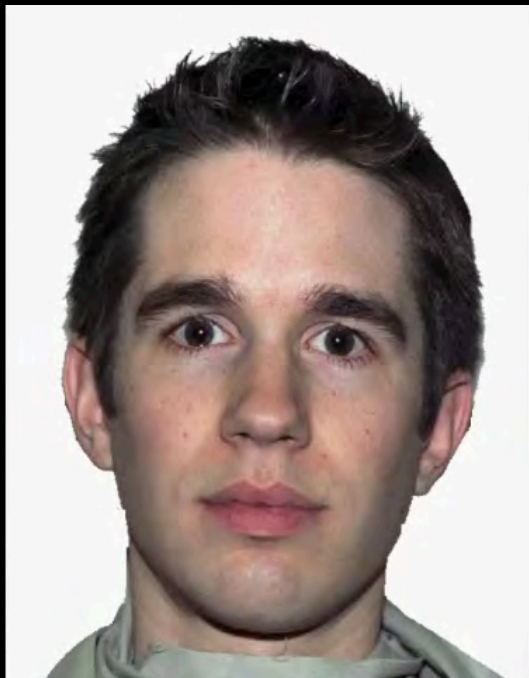
- 9 participants with ASD, 7 with treatment and 2 without
- Age at first exam: 19.3 ± 4.6
- Second exam after 10 months of bumetamide treatment (1mg/day)
- Behavioral and fMRI testing at each exam

Behavioral study – RT and accuracy

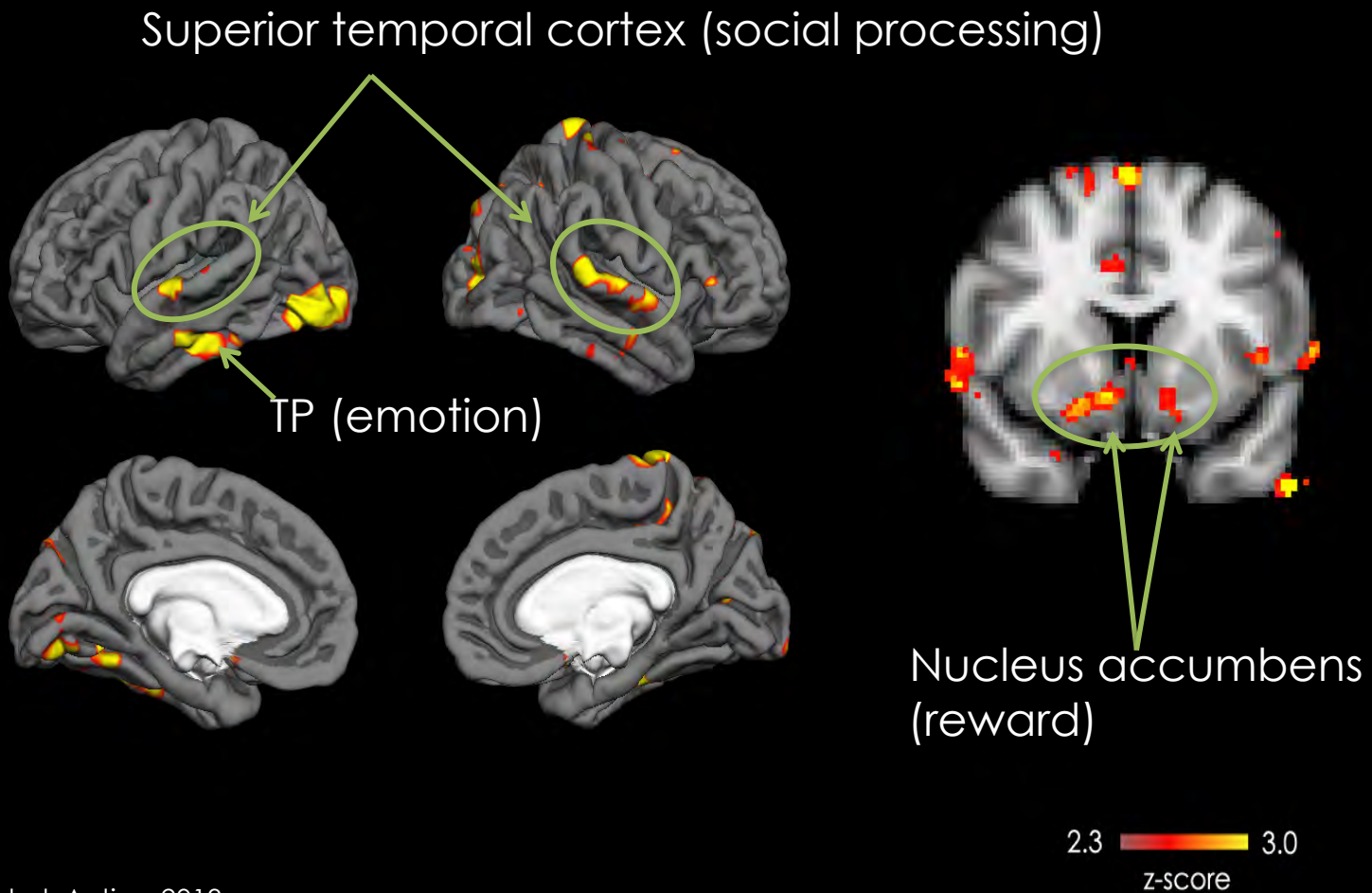


Non verbal emotion matching,
morph at 40% intensity

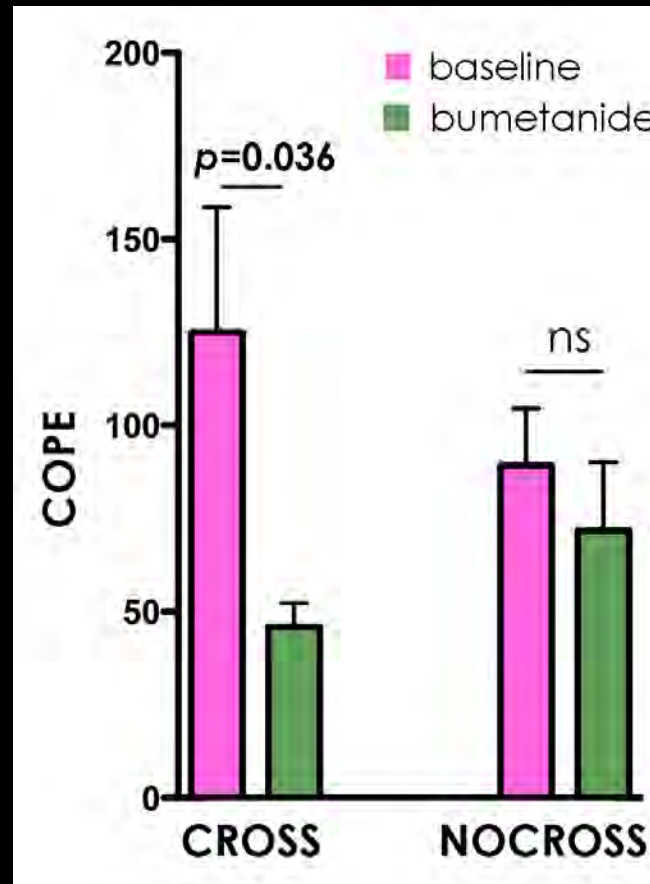




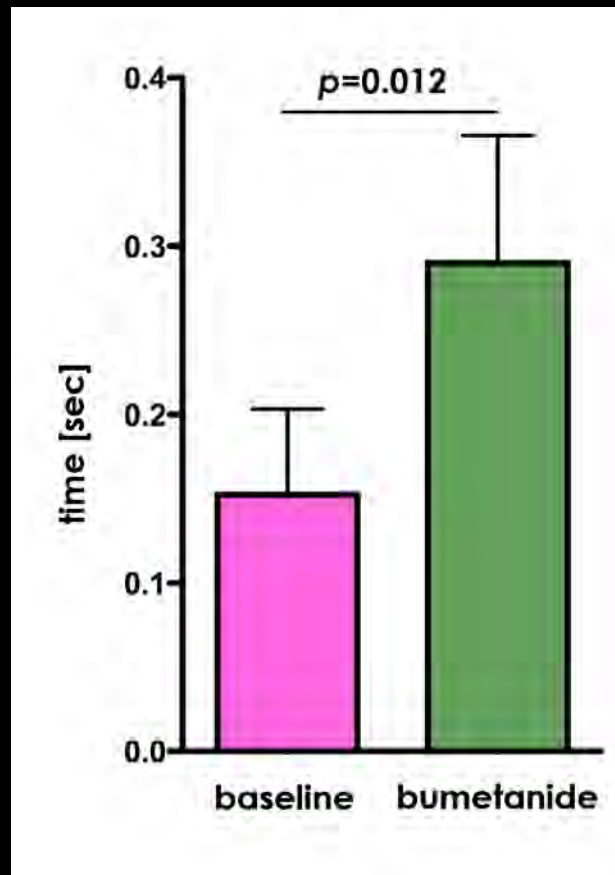
$[EMO > NEU] \text{ POST}_{\text{bumet}} > [EMO > NEU] \text{ PRE}_{\text{bumet}}$



Amygdala activation: eye-contact effect normalizes after bumetanide



Spontaneous looking in the eyes is increased



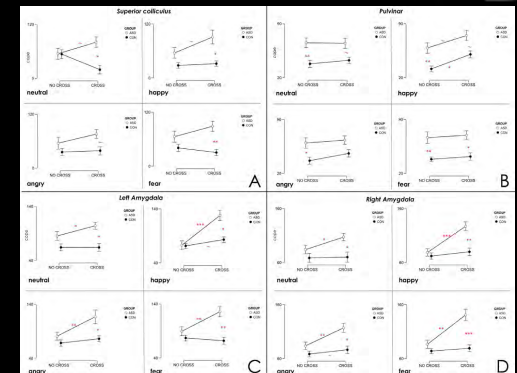
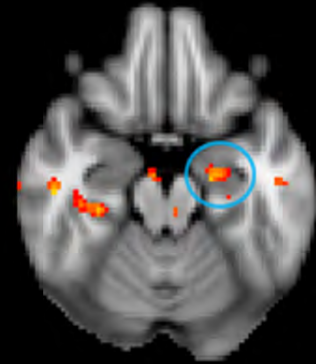
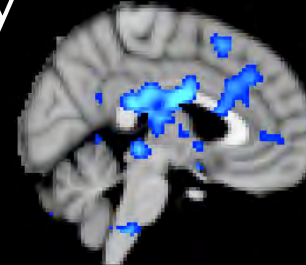
Bumetanide

- Novel, safe and **etiology-driven** therapy available for a large proportion of ASD.
- The guiding hypothesis is that although a variety of genetic and environmental insults are linked to ASD, **disturbed chloride homeostasis is a common contributing mechanism to pathological brain activity** in ASD and can be treated with the chloride transporter antagonist bumetanide.
- This therapy is safe, given the extensive experience with this drug as a diuretic treatment in children and adults. Side effects are well known: **hypokaliemia and enuresis**.
- In contrast with other existing treatments, the application of bumetanide is etiologically driven and **will not affect the central nervous system in neurons in which chloride homeostasis is unaffected**.

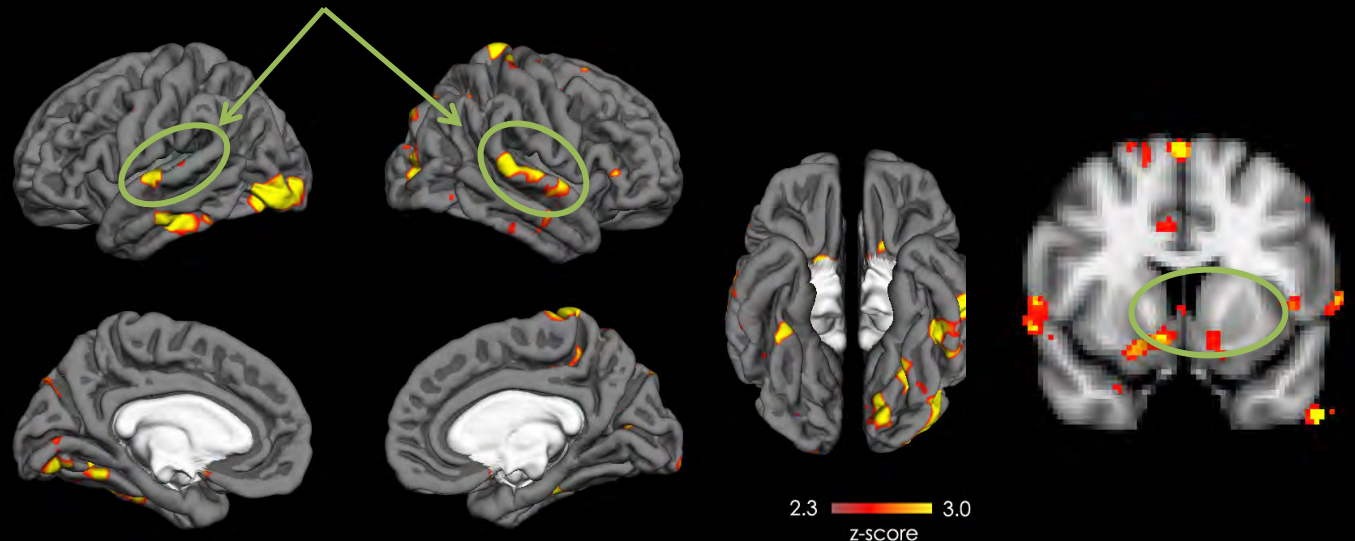
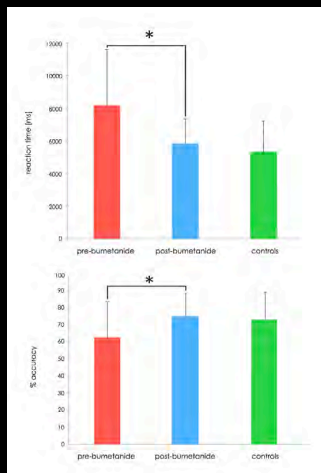
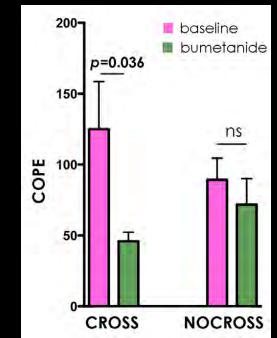
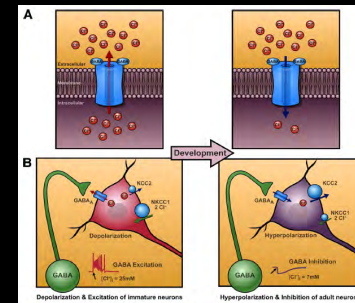
Conclusions

There are evidence of abnormalities in the subcortical pathway

- Abnormal activation in response to gaze cues of danger
- Hypersensitivity to low intensity fearful faces
- Hyperactivation of the subcortical pathway when constrained to look at the eyes



- Treatment with a diuretic restoring GABA function seems to significantly improve behavior and function



This, and other data support the E/I imbalance hypothesis in ASD

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attention!

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