OXYTOCIN – CURRENT STATUS & QUESTIONS.

What are the functions of oxytocin, and the related peptide, vasopressin?

How can these be measured?

To what extent are these sexually dimorphic?

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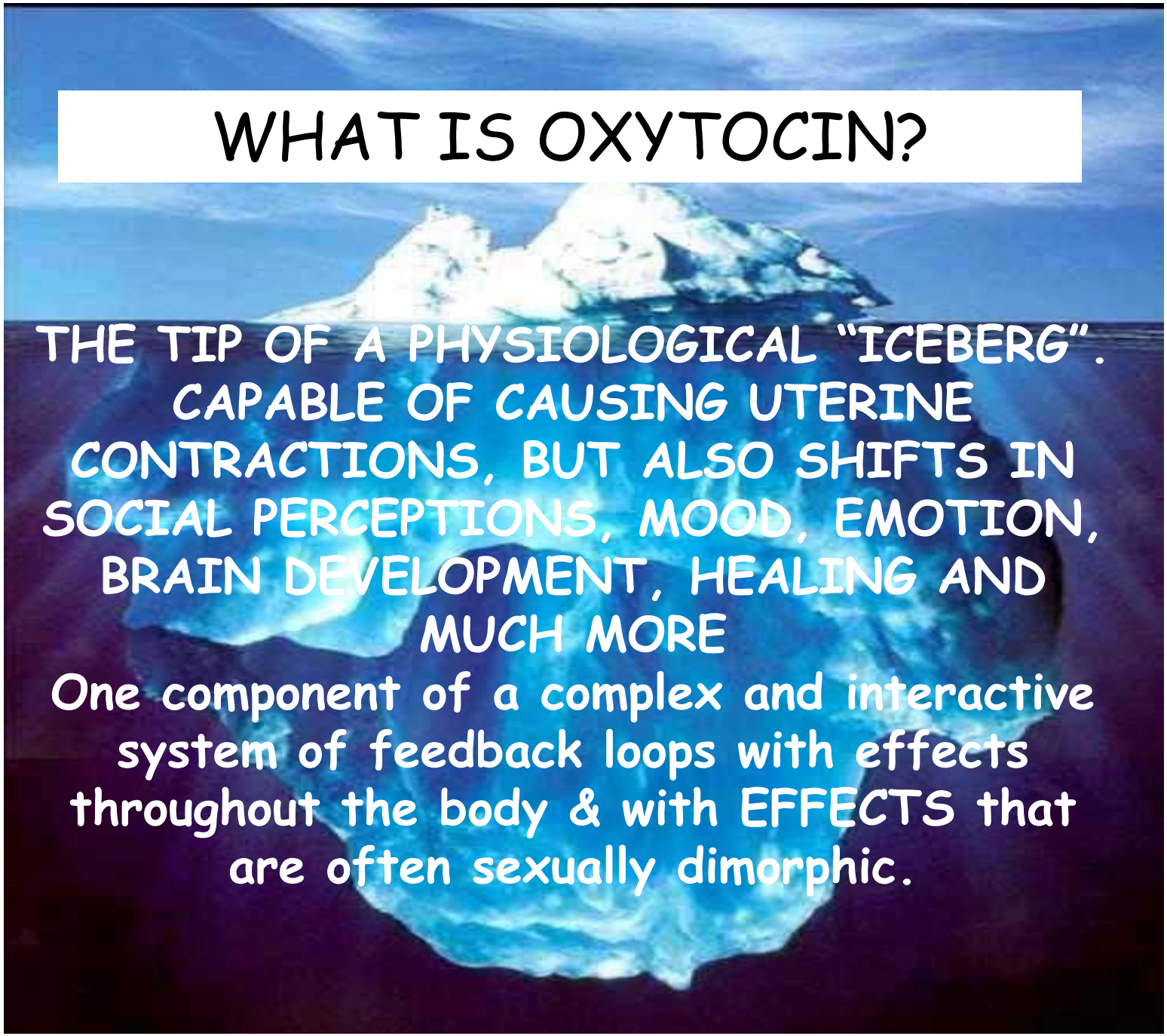
I declare that I have no conflicts of interest.

The research described here was conducted with support from the NIH, including P01 HD 075750, "The developmental consequences of birth interventions"

WHAT IS OXYTOCIN?

A PHYSIOLOGICAL METAPHOR for SAFETY??

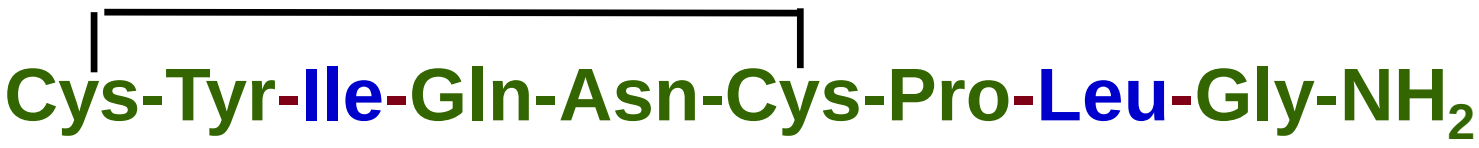
WHAT IS OXYTOCIN?



THE TIP OF A PHYSIOLOGICAL "ICEBERG". CAPABLE OF CAUSING UTERINE CONTRACTIONS, BUT ALSO SHIFTS IN SOCIAL PERCEPTIONS, MOOD, EMOTION, BRAIN DEVELOPMENT, HEALING AND MUCH MORE

One component of a complex and interactive system of feedback loops with effects throughout the body & with EFFECTS that are often sexually dimorphic.

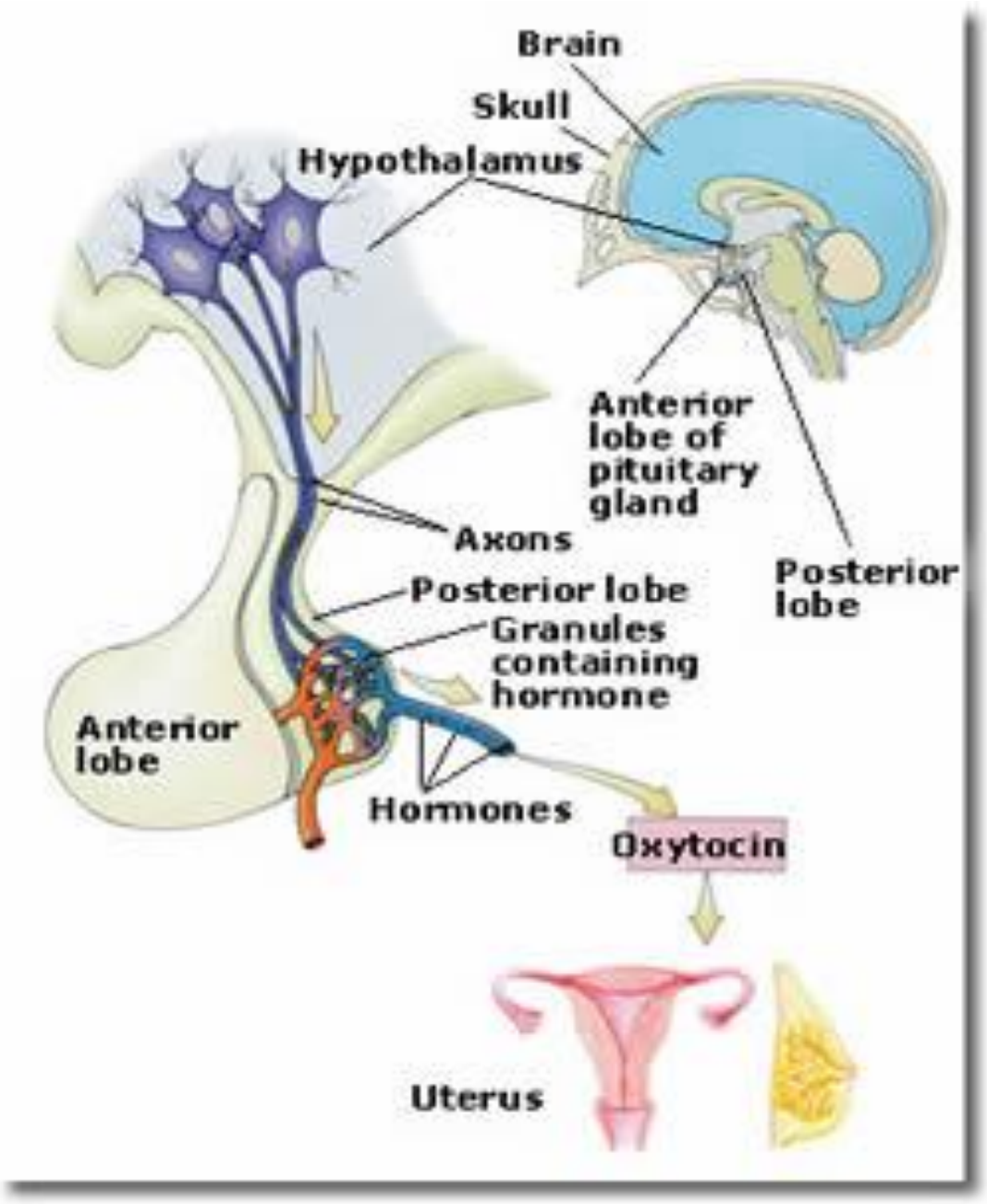
OXYTOCIN

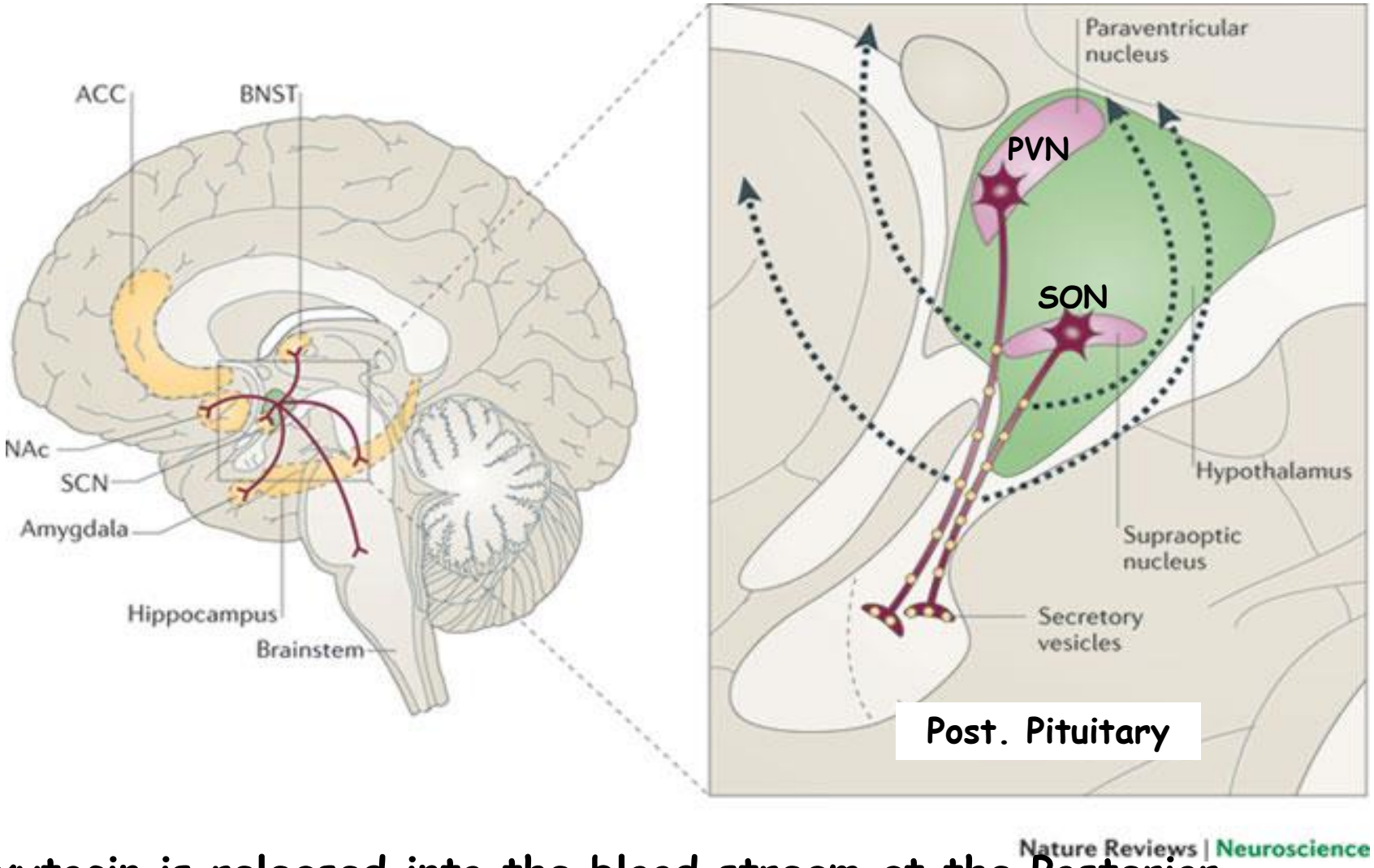


9 amino acids configured as a ring and a tail

Oxytocin was classically viewed as a "FEMALE REPRODUCTIVE" Hormone, Acting primarily On the UTERUS And MAMMARY GLAND.

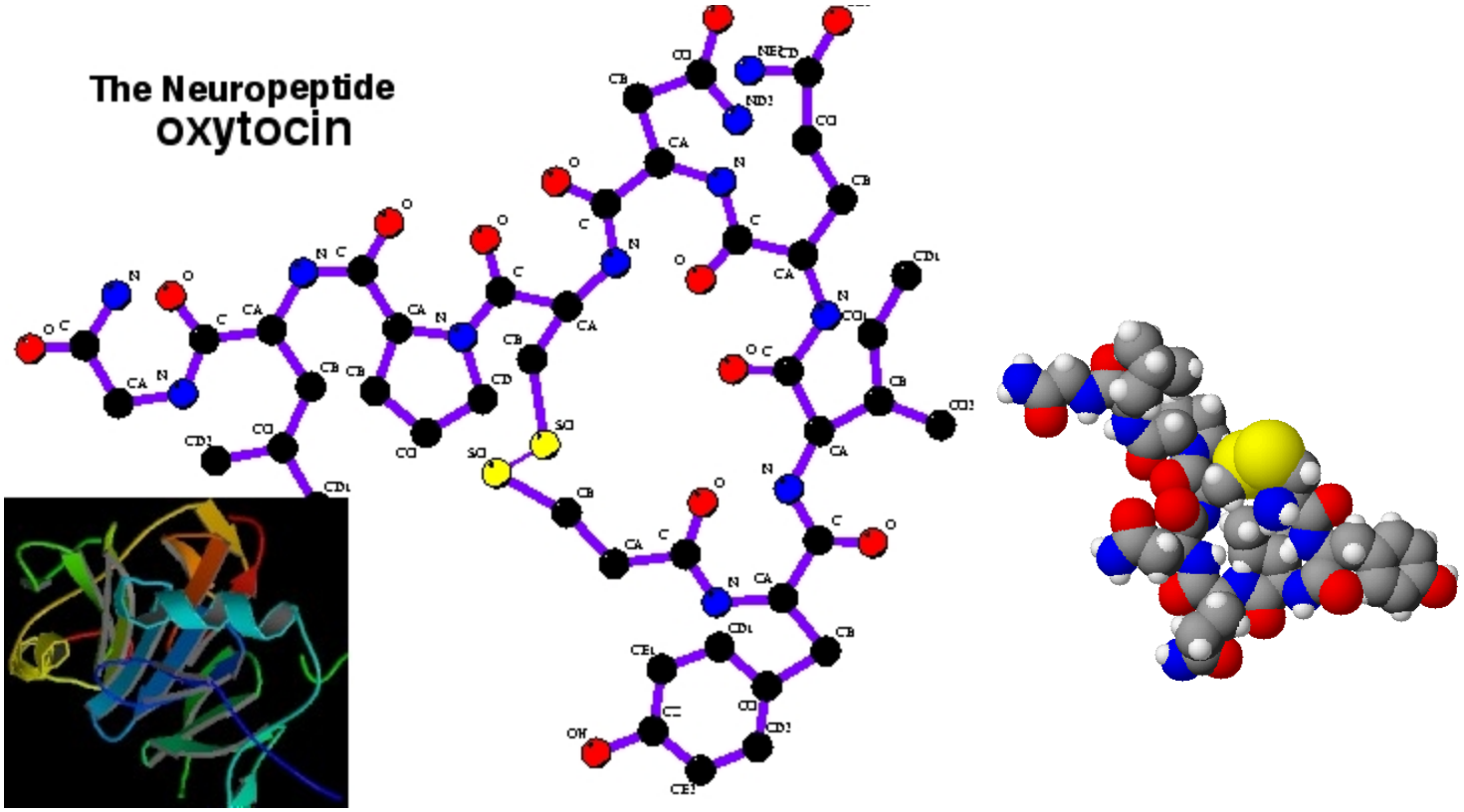
This is only part of the story!



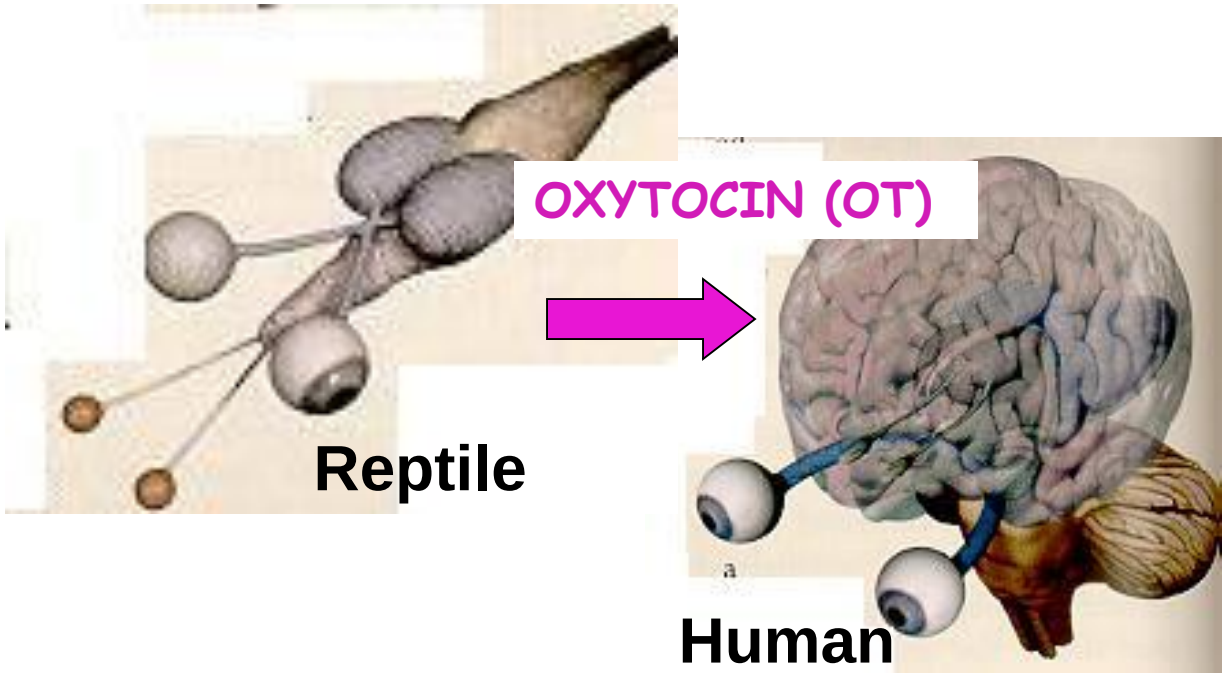


Oxytocin is released into the blood stream at the Posterior Pituitary, but is also released within the nervous system. Oxytocin can affect social behavior, the autonomic nervous system and the immune system,allowing the body to ADAPT, protect and heal itself in the face of challenge.

Oxytocin is not only central to the biology of social behavior, social bonds, social support, sexual behavior but also may have permitted the EVOLUTION OF THE HUMAN NERVOUS SYSTEM.



The human nervous system is a consequence of evolution, with a massive increase in the cerebral cortex



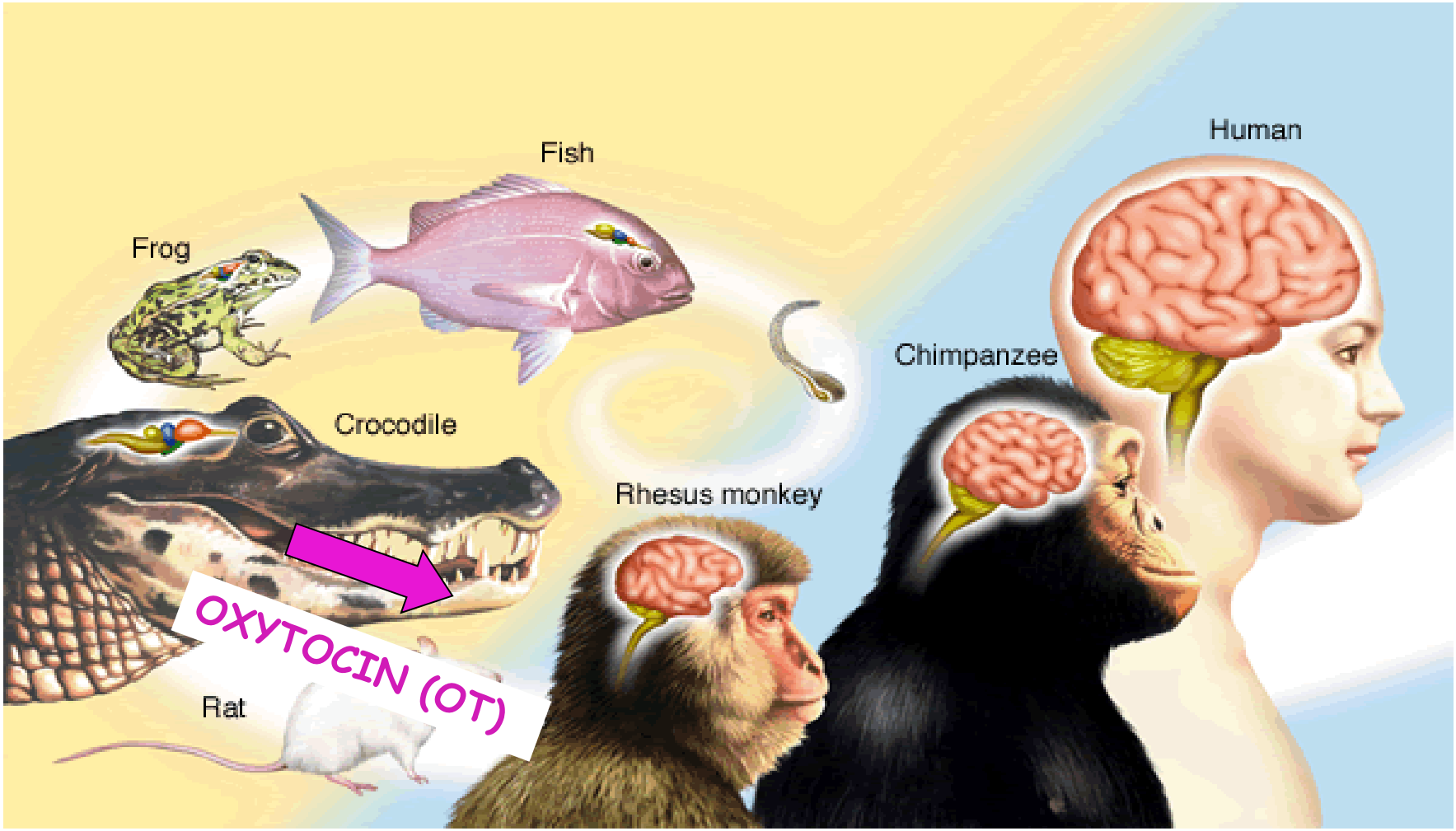
OXYTOCIN allows the transition from reptile to mammal.

OT permits birth (helps expel the large-brained baby from the uterus)

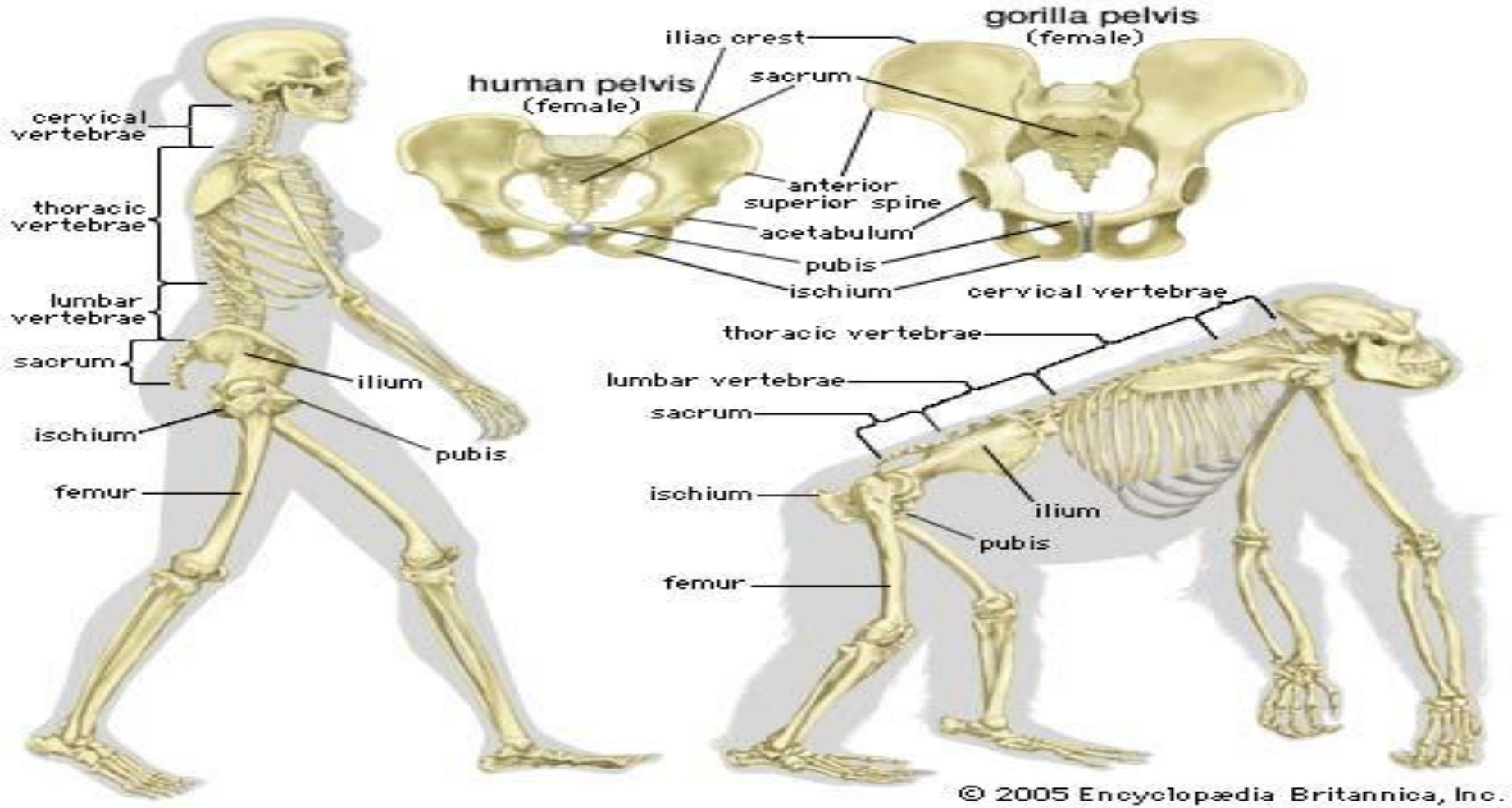
OT permits post-birth nutrition & supports the baby (lactation/maternal behavior/alloparenting)

OT facilitates oxygenation of the brain (myelinated vagus).

PERMITS HUMAN COGNITION AND SOCIAL BEHAVIOR!



OXYTOCIN permitted the EVOLUTION of the MAMMALIAN NERVOUS SYSTEM and eventually the EVOLUTION of the HUMAN NERVOUS SYSTEM and the COGNITIVE EXPERIENCES WE CALL "LOVE"



HUMAN BIRTH presents special problems because of our

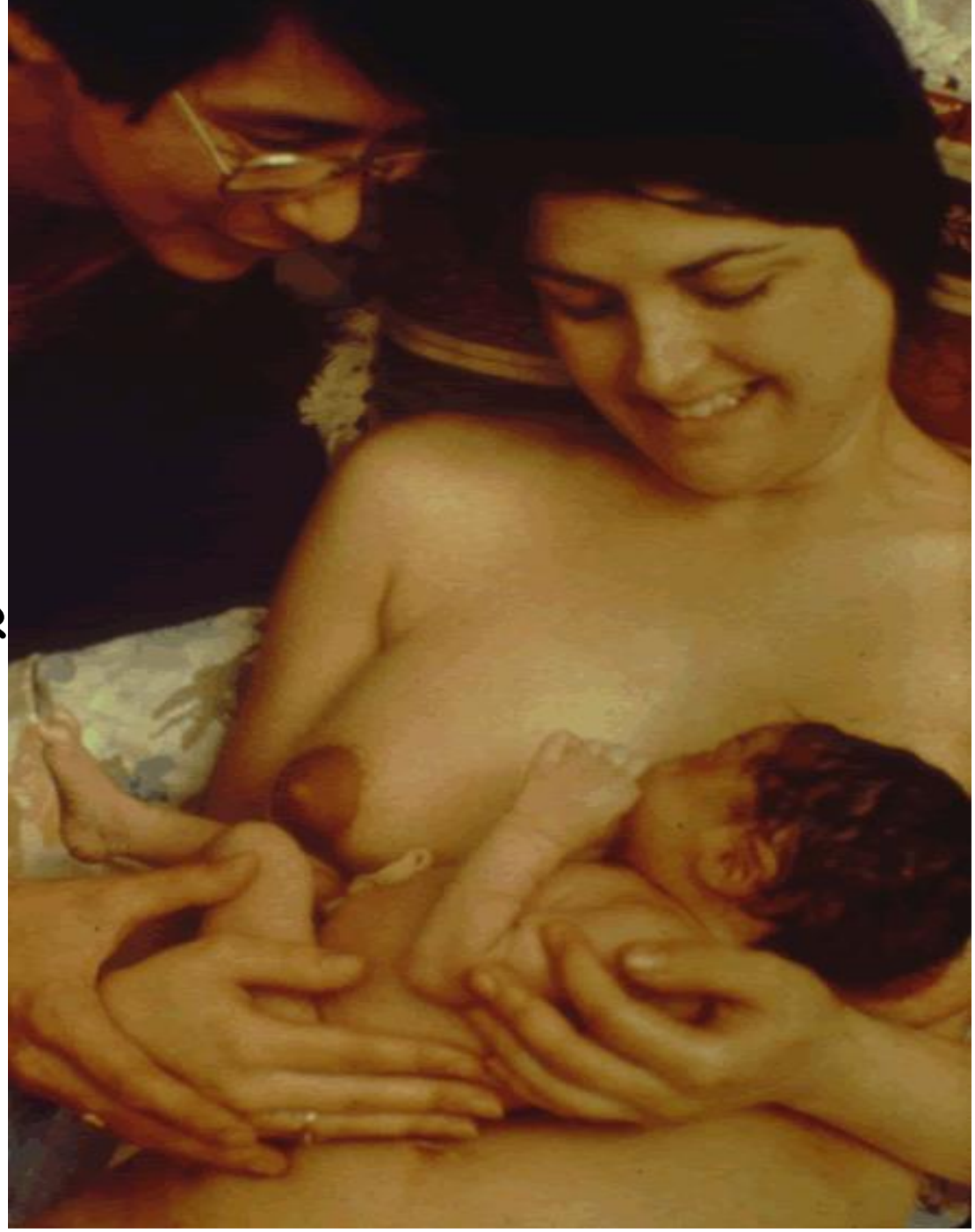
- **BIG CORTEX and SKULL,**
- **BIPEDAL LOCOMOTION and**
- **SMALL, RIGID PELVIC GIRDLE**

OXYTOCIN FACITATES BIRTH by STRONG UTERINE CONTRACTIONS


Oxytocin helps to expel the
large headed baby during birth

Oxytocin supports or permits:

PREGNANCY
INFANT NUTRITION
MATERNAL BEHAVIOR
PATERTAL BEHAVIOR
ALLOPARENTAL BEHAVIOR
and
EXTENDED NURTURE
of the
immature
human offspring



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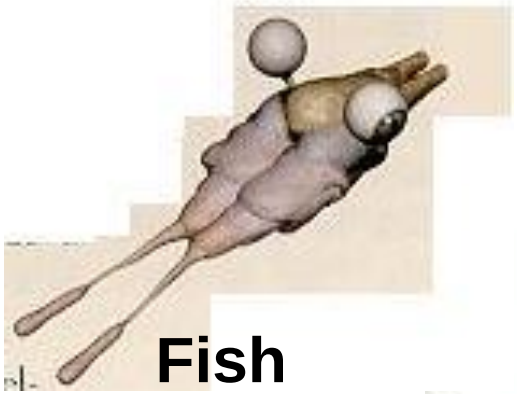
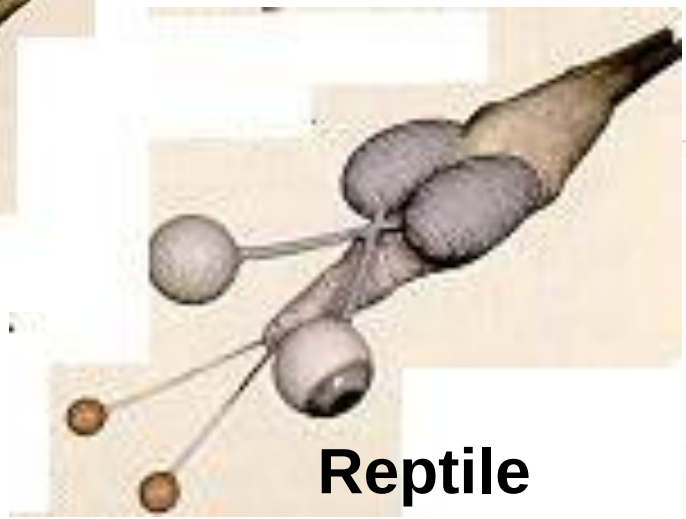
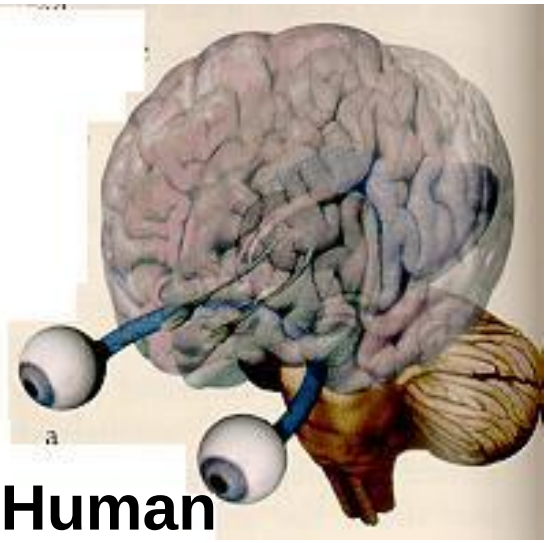


Fish



Reptile



Human

But, older parts of the nervous system are still represented, and can influence the actions of more modern components. Much of the wiring comes UP from the body and lower brain regions, with fewer pathways that come down. This is why it is hard to use cognition to control emotion. THIS IS ONE REASON THAT OXYTOCIN IS SO POWERFUL IN REGULATING STATES AND EMOTIONS.

Oxytocin is a neuropeptide hormone/neuromodulator…

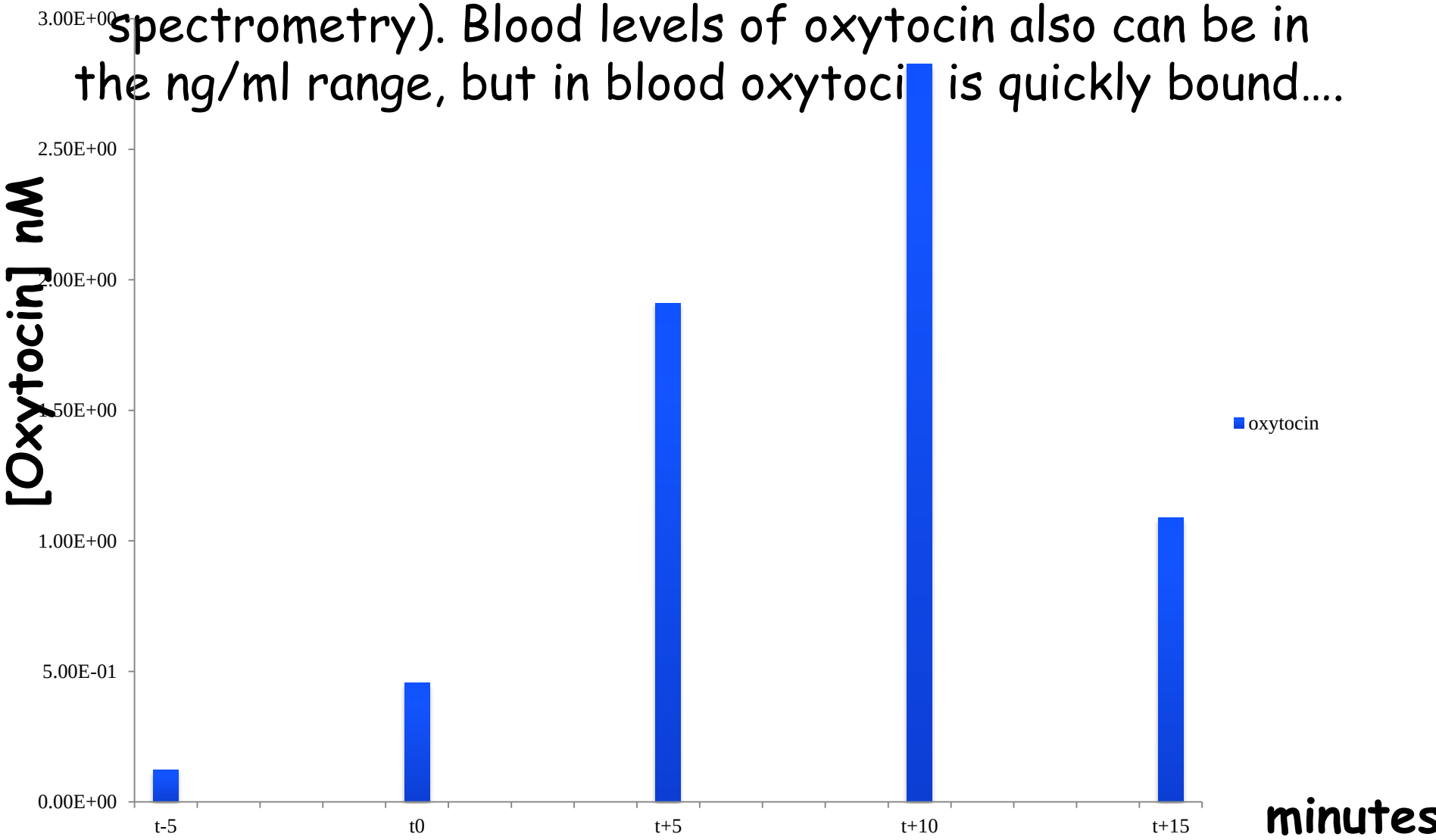
1. made primarily in the brain (hypothalamus) & released into the blood supply at the posterior pituitary from which is acts on the uterus (birth) and Mammary tissue (milk ejection)
2. also released into the brain & spinal cord where it binds to OXYTOCIN receptors OTRs) to influence behavior & physiology
3. possibly a major factor in the body's capacity to PROTECT or HEAL in the face of either emotional or physical challenge/stress.
4. evidence for HEALING – partial list
   - injured skin (burns),
   - heart (cardiac infarct)
   - bone (osteoporosis)
   - intestines (intestinal bowel disease)
   - brain (stroke)
   - mental disorders (anxiety, depression autism, schizophrenia)
5. Oxytocin is an anti-inflammatory and anti-oxidant
6. May explain effects of probiotics, such as L. reuteri

Research tools available for mapping OXYTOCIN PATHWAYS:

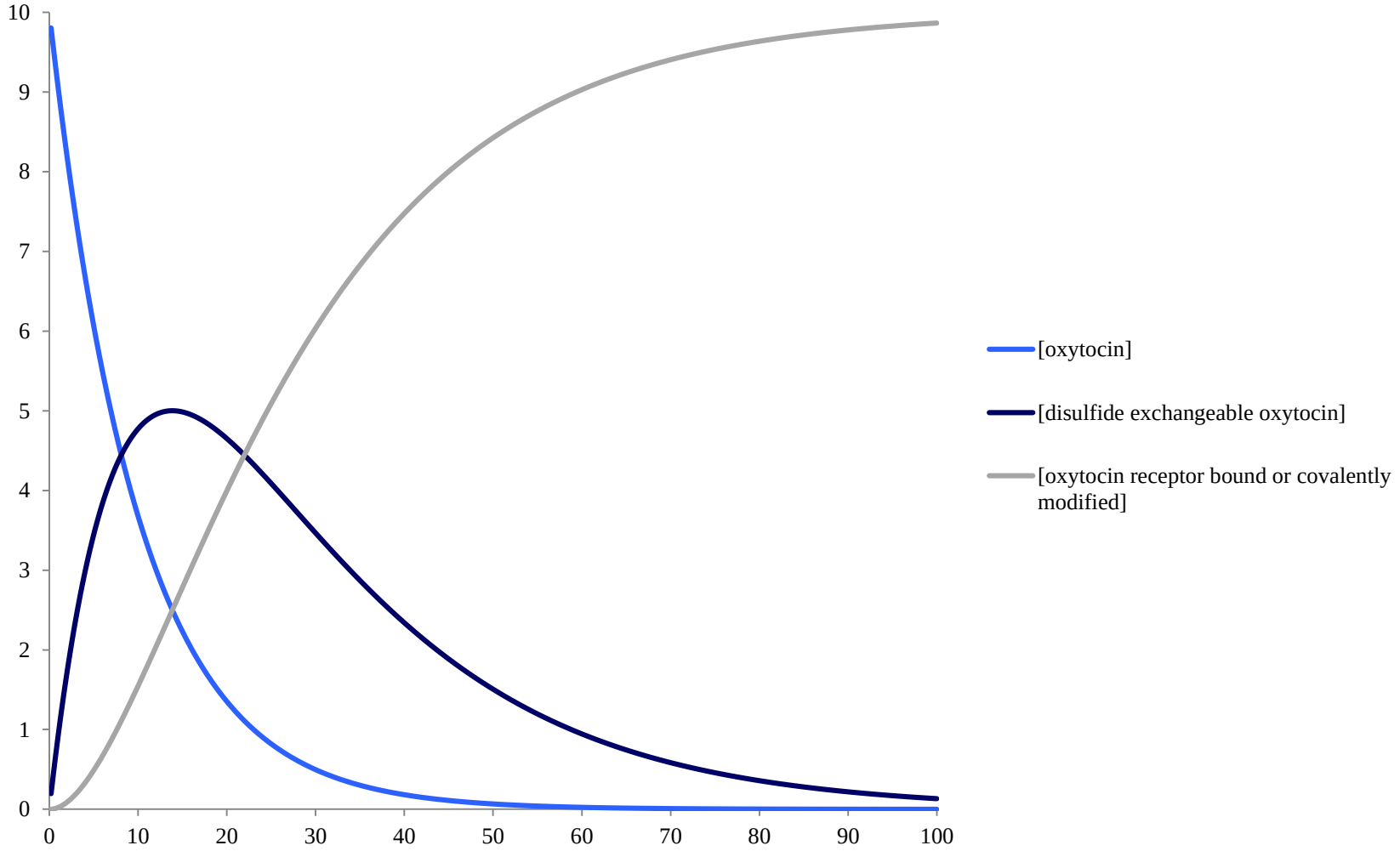
1. The prairie vole model, which shares with humans
    a. High sociality and pair bonds
    b. Responses to social isolation & breaking bonds
    c. High levels of parasympathetic/vagal tone (RSA)
    d. High levels of OXYTOCIN
    e. Homologous patterns of methylation of the OXTR (not found in mice or rats, possibly related to somewhat unique sociality of the prairie vole?)
    f. Sex differences in sociality & responses to early experience

2. Tools for measuring OXYTOCIN PATHWAYS
    a. Oxytocin and vasopressin mass spec assay (Martin Protean)
        Or Enzyme-Immuno Assays (Enzo & others)
    a. Oxytocin receptor methylation as a possible proxy/marker for the activity of the OXYTOCIN RECEPTOR (Jessica Connelly)
    b. Autonomic measures, including non-contact polygraphy and unique measures of RSA (vagal tone). OT may act on autonomics to influence mood, food intake, healing, etc.

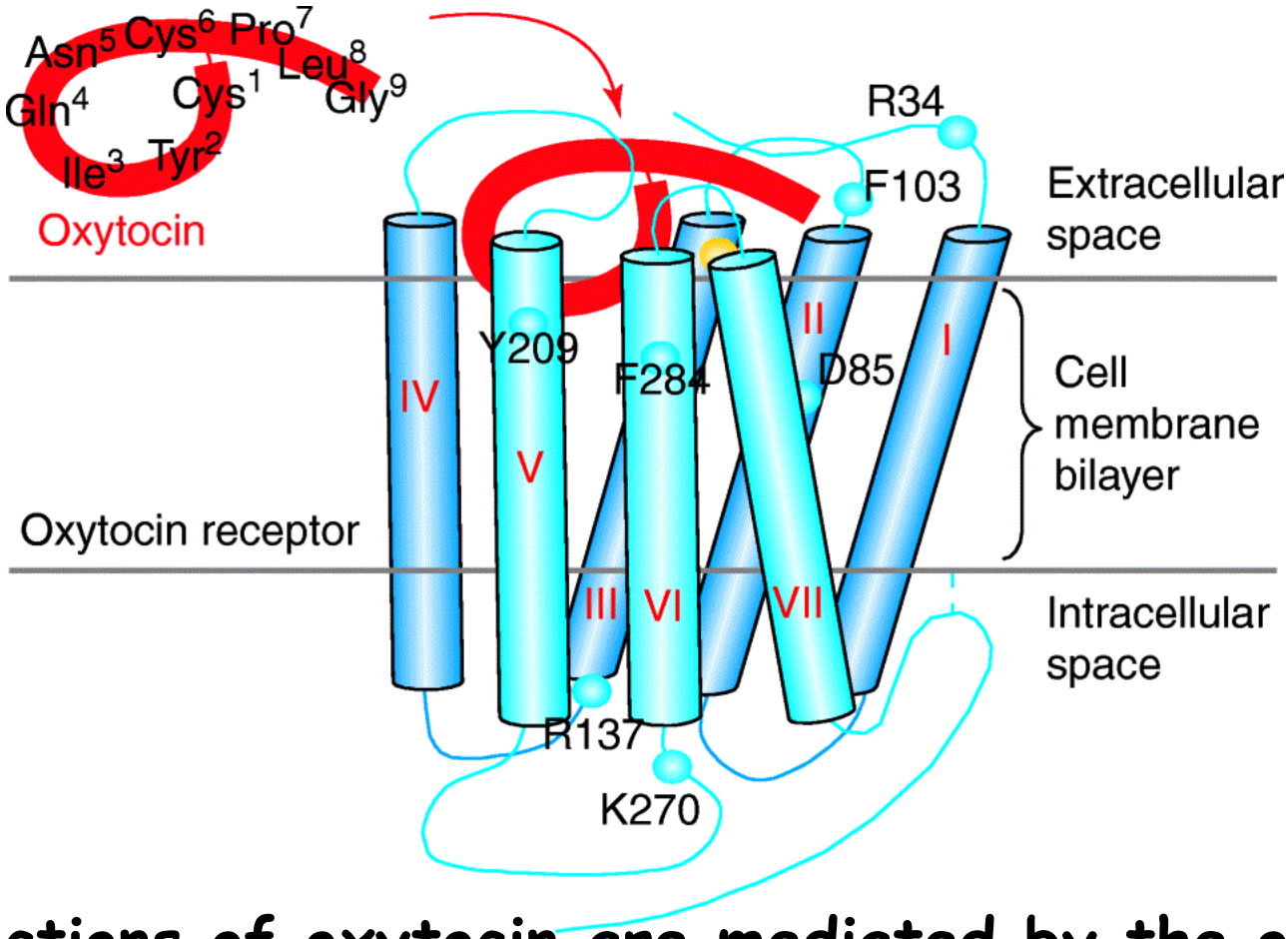
Oxytocin can be measured in saliva, and can reach levels near or above 1 ng/ml (1000 pg/ml) within 10 min – for example following orgasm (measured by mass spectrometry). Blood levels of oxytocin also can be in the ng/ml range, but in blood oxytocin is quickly bound….



Oxytocin is difficult to measure in blood because it changes states quickly (within minutes) from molecules that are in forms that are
(a) free unbound (blue) to
(b) disulfide/loosely bound (purple) to
(c) to receptor bound/covalent (grey)
(model from Lance Martin; Martin Protean. LLC)



Oxytocin Receptor Signaling



The actions of oxytocin are mediated by the oxytocin receptors (OXTR), which are found throughout the body including the central nervous system.

From: Zingg and Laporte . *TRENDS in Endocrinology and Metabolism (2003)* 14(5): 222-227

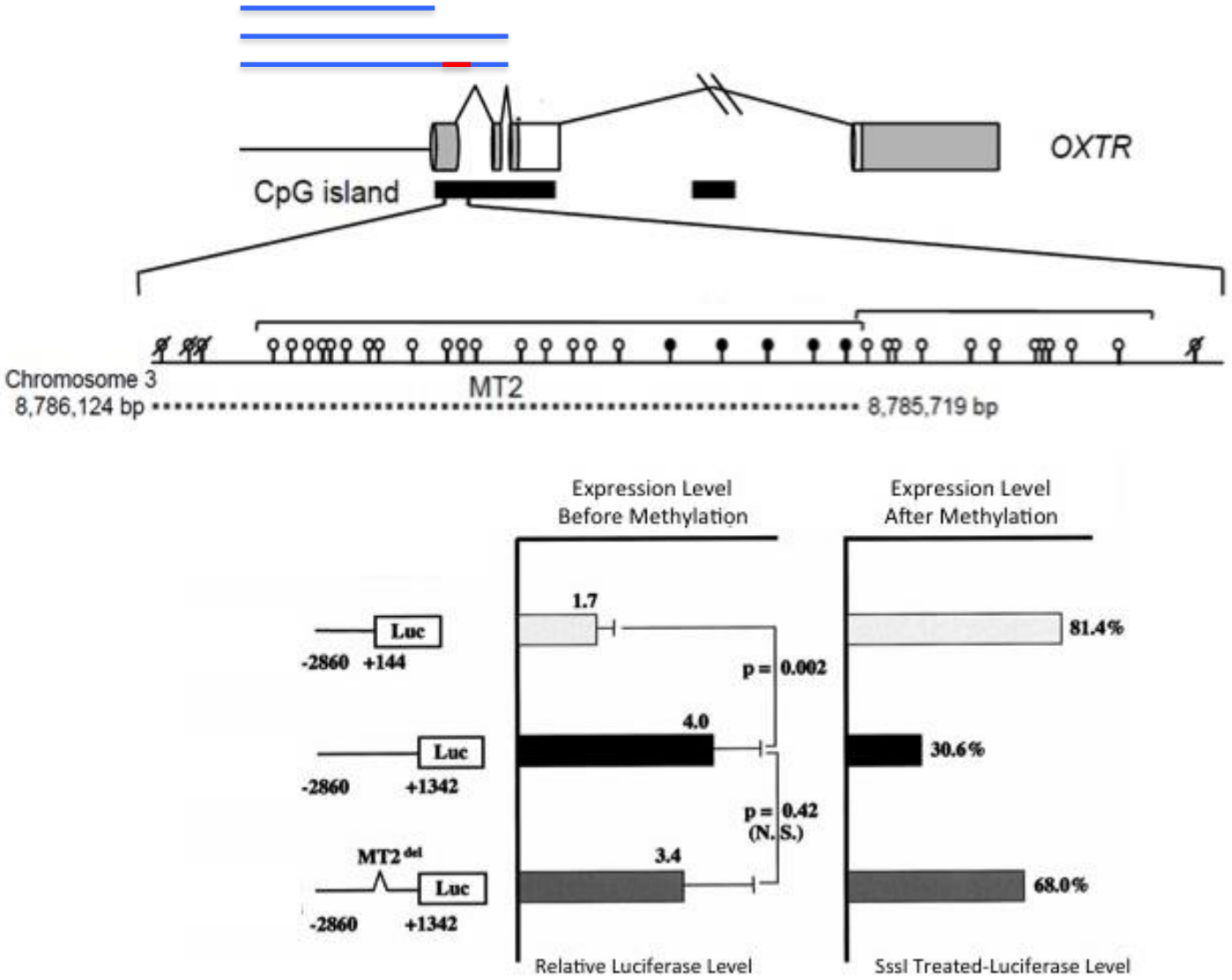
Novel methods for measuring the methylation (silencing) of the OXTOCIN RECEPTOR (OXTR) were developed by Jessica Connelly (U Va).

These show that:

1. Methylation of the OXTR gene is associated with lower levels of gene expression for the oxytocin receptor.
2. OXTR methylation is higher in autism.
3. OXTR methylation is significantly related to brain activation (fMRI) in response to social stimuli (animacy).
4. OXTR methylation (hi level) is associated with postpartum depression (but only in women carrying the GG allele of rs53576).
5. OXTR methylation is increased by early OT exposure in FEMALE, but not in male prairie voles.
6. The GENETICS and EPIGENETICS of the OXTR, TAKEN TOGETHER, may predict SENSITIVITY TO THE OXYTOCIN PEPTIDE, and the CAPACITY OF THE BODY to respond to OXYTOCIN.

Jessica Connelly

DNA methylation controls expression of *OXTR*

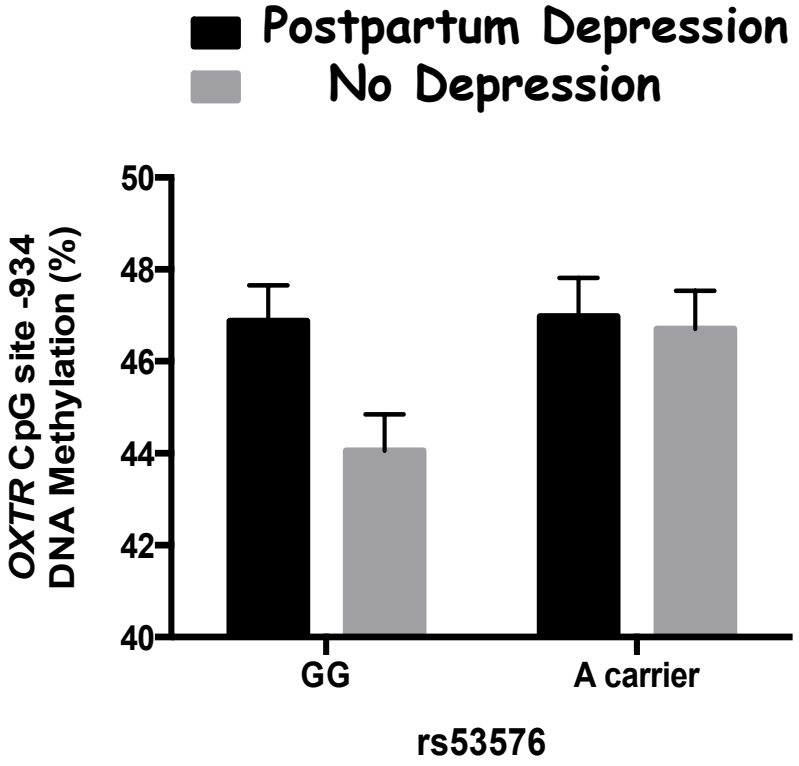


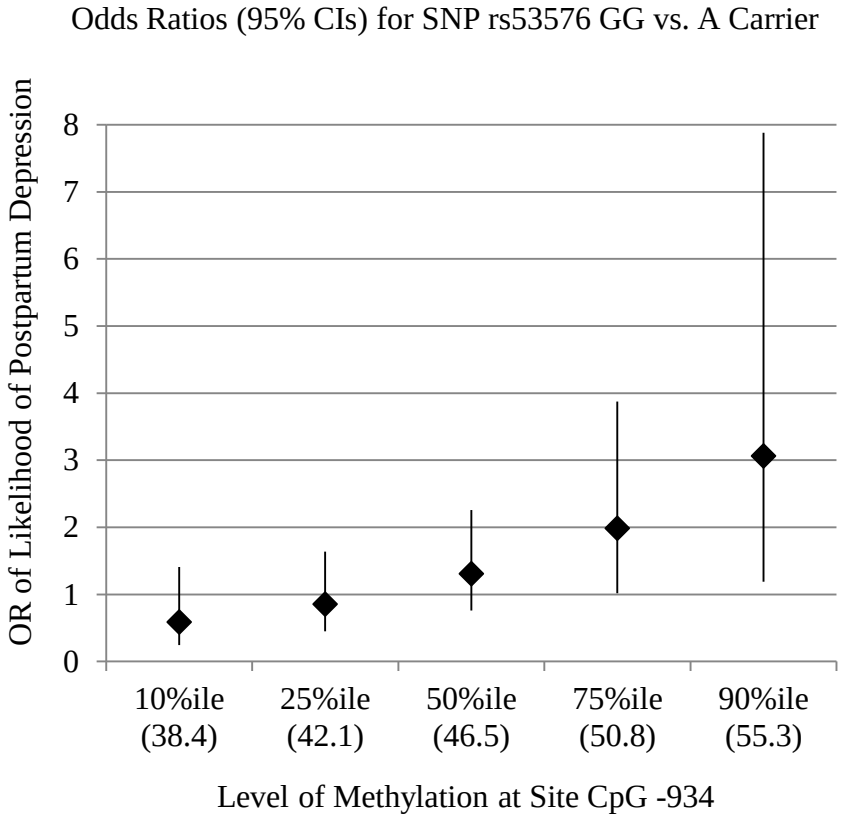
A region of *OXTR* termed MT2 contains a cluster of CpG sites that when methylated partially control expression of the gene.

From: Kusui et al. *Biochemical and Biophysical Research Communications (2001)* 289: 681-686

Women who experience postpartum depression exhibit increased methylation (gene silencing) at *Site CpG -934 in the OXTR, in those with rs53576GG*

ALSPAC study (n=576): EPDS >13 PPD





Bell, A.F., Carter, C.S., Steer, C.D., Golding, J., Davis, J.M., Steffen, A.D., Rubin, L.H., Lillard, T.S., Gregory, S.P., Harris, J.C., & Connelly, J.J.
Frontiers in Genetics 2015

Intranasal spray interventions treating with EXOGENOUS OXYTOCIN have been used successfully in dozens of studies in humans and other animals..

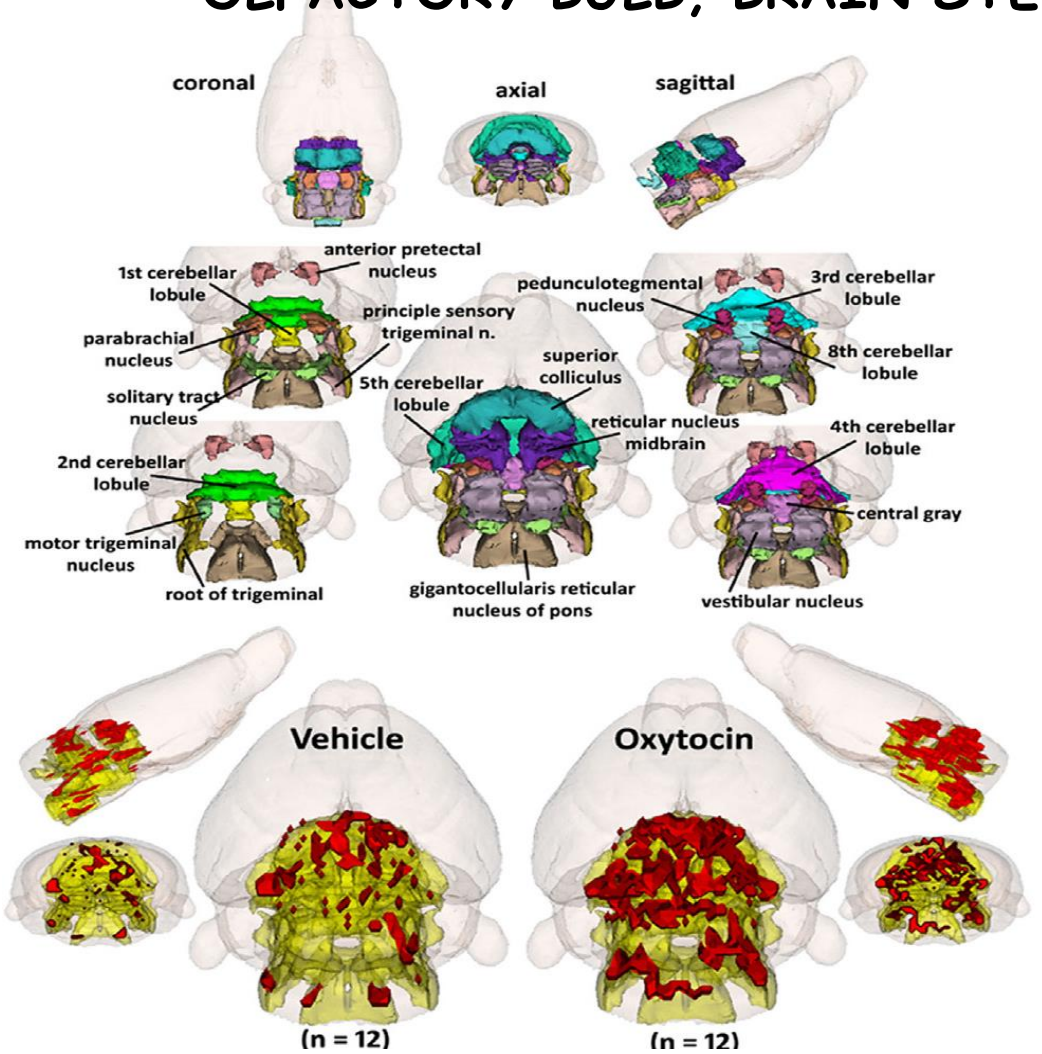
It works - But where and how?

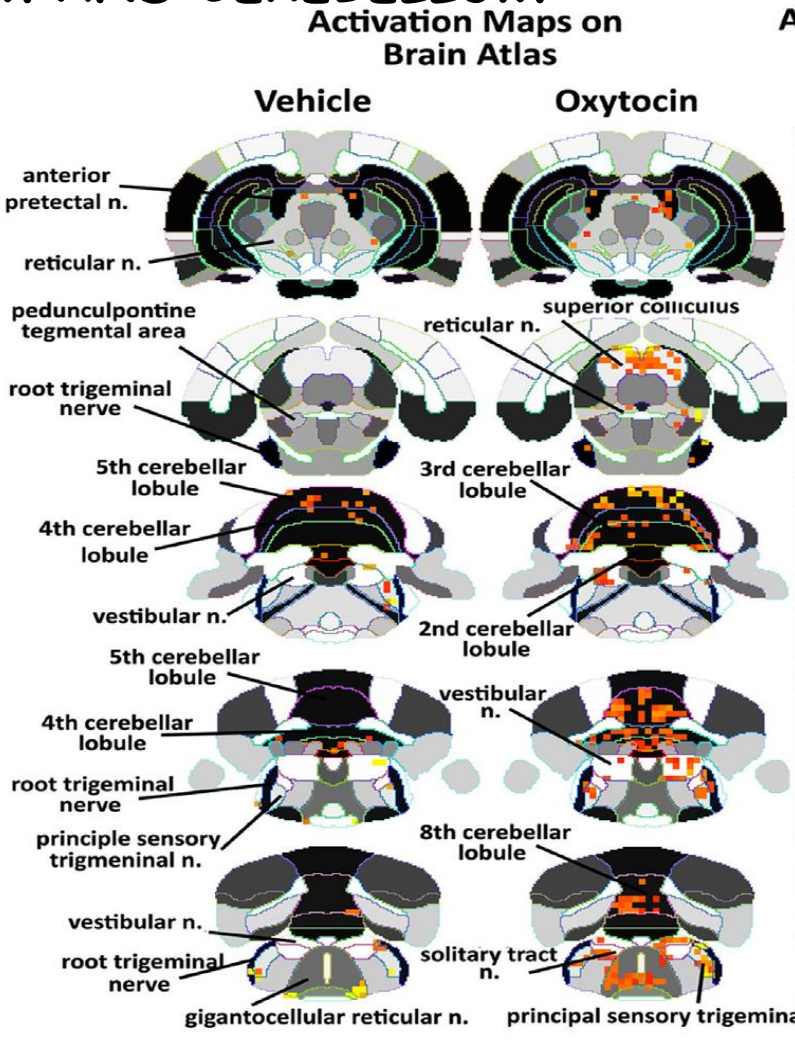
Brain?

Autonomics?

Other?

Oxytocin given peripherally (IP) activates OLFACTORY BULB, BRAIN STEM AND CEREBELLUM





Front. Behav. Neurosci., 17 September 2015. Distinct BOLD activation profiles following central and peripheral oxytocin administration in awake rats.
C. F. Ferris, J, R. Yee, W. M. Kenkel, K.M. Dumais, K. Moore, A. H. Veenema, P, Kulkarni, A. M. Perkeybile, & C. S. Carter

The effects of oxytocin are most easily understood in the context of evolution. Most positive social behaviors directly or indirectly support:

SURVIVAL

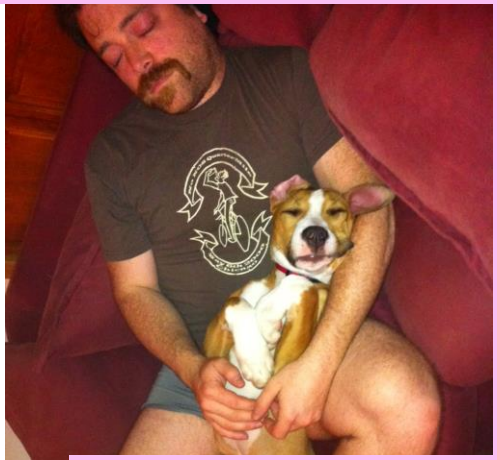
- social support & safety

REPRODUCTION

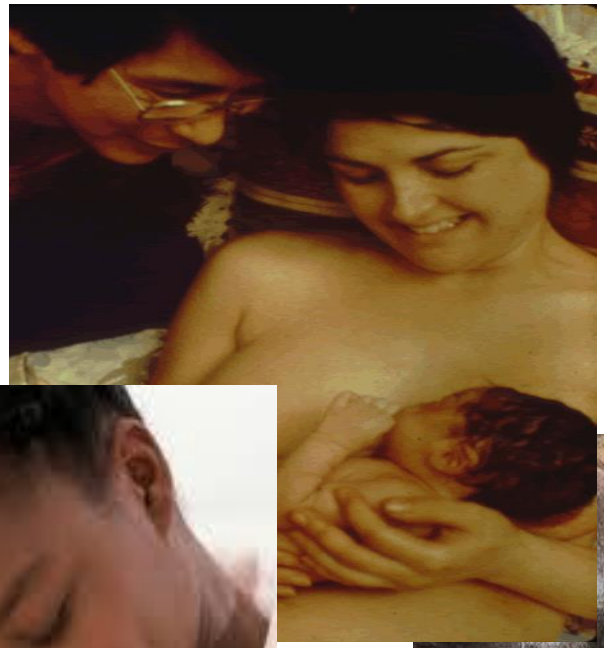
- access to mates
- care of offspring
- genetic survival

Oxytocin may be released under conditions that involve positive social interactions, including:

Social support is critical to human reproduction and survival can come from various sources including partners, children and pets.



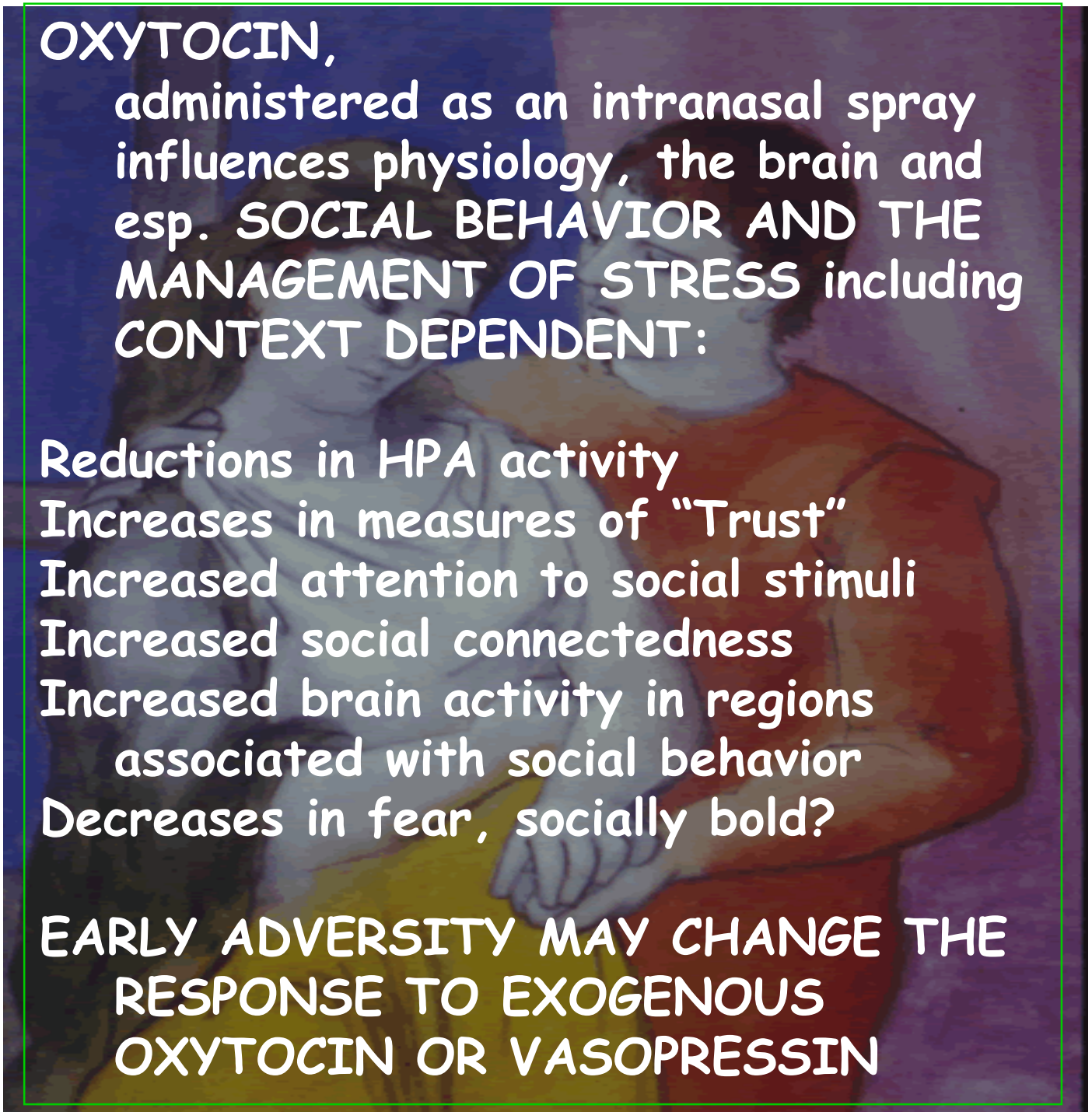
Social behaviors are not "one-way streets". The best relationships are reciprocal



The EVOLUTIONARY prototype for Positive social behaviors in mammals is the mother-child interaction

Lactation may allow a new mother to manage stress more effectively.Less reactivity or more appropriate reactions to stressors, including stressful stimuli associated with child rearing.

NURSING and the physiology of lactation are a buffer between the physiological state of pregnancy & the postpartum period - in part through effects of OXYTOCIN.



OXYTOCIN,

administered as an intranasal spray influences physiology, the brain and esp. SOCIAL BEHAVIOR AND THE MANAGEMENT OF STRESS including CONTEXT DEPENDENT:

Reductions in HPA activity
Increases in measures of "Trust"
Increased attention to social stimuli
Increased social connectedness
Increased brain activity in regions associated with social behavior
Decreases in fear, socially bold?

EARLY ADVERSITY MAY CHANGE THE RESPONSE TO EXOGENOUS OXYTOCIN OR VASOPRESSIN

Oxytocin does not act alone – for example, OXYTOCIN has a sibling hormone - VASOPRESSIN - from which it differs by 2 (of 9) amino acids

OXYTOCIN (OT)

Arginine VASOPRESSIN (AVP)

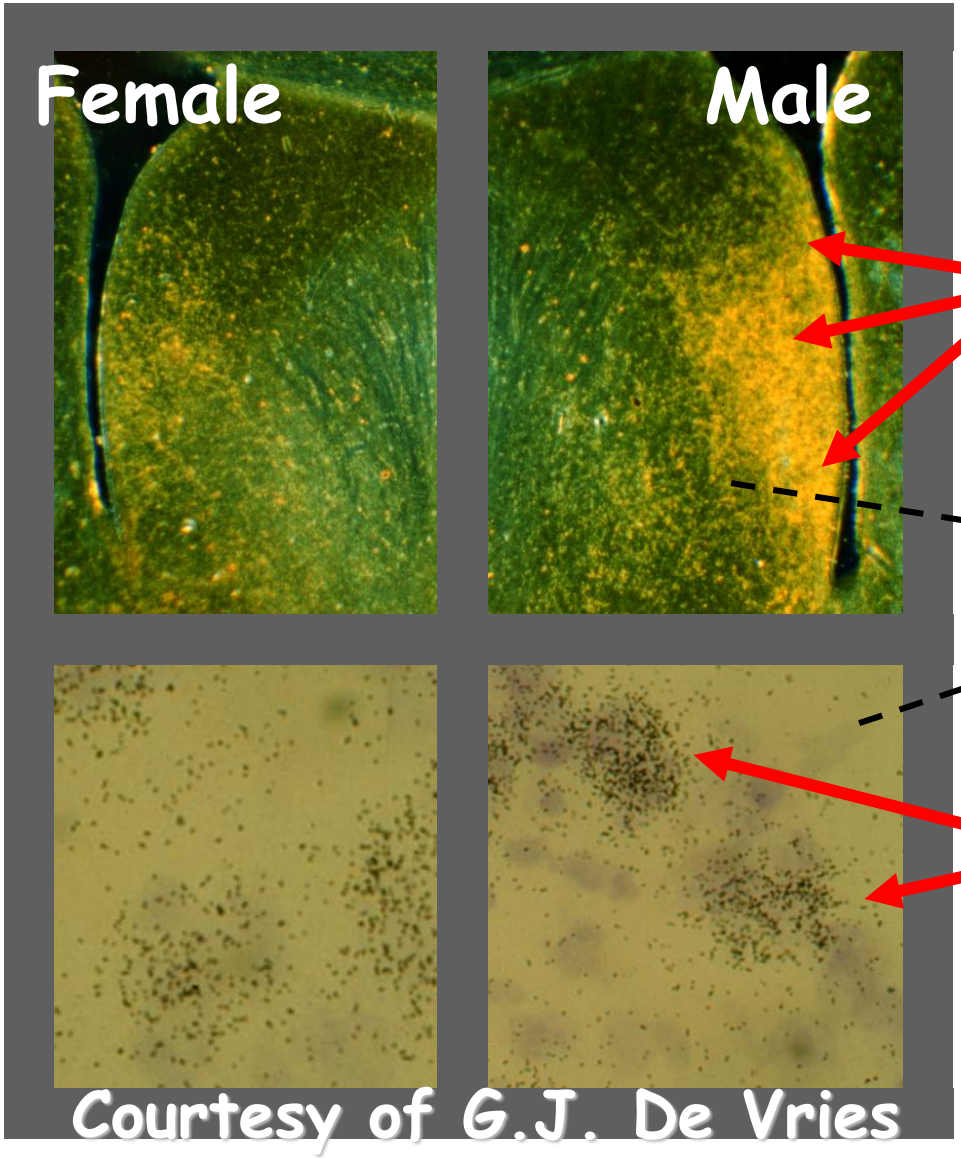
The EVOLUTION OF HUMAN SOCIALITY involves:

A dynamic dance between
Oxytocin and Vasopressin
(both of which are critical to mental health)

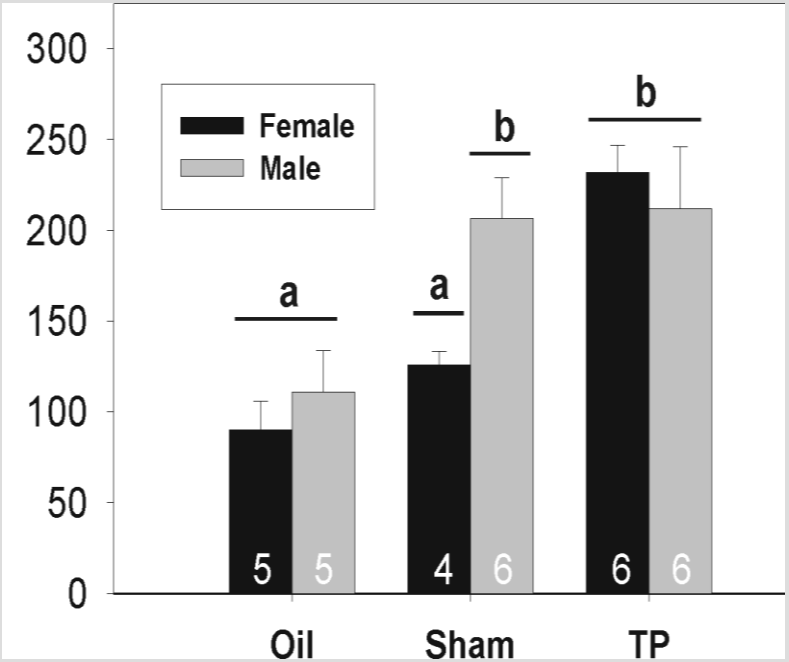
Oxytocin facilitates SOCIAL ENGAGEMENT
And a Sense of Safety
(love, empathy, compassion, relaxation)

Vasopressin may allow DEFENSE
OF SELF AND OTHERS
(vigilance, hypermobilization, territoriality, arousal)

Vasopressin projections in the Lateral Septum (from the extended Amygdala) are denser in MALES than in females.



Neonatal testosterone increases the number of BST cells that express AVPmRNA



Oxytocin and Vasopressin evolved from an ancestral molecule, that preceded the evolution of Vertebrates, but have different actions:

PEPTIDE:	***OXYTOCIN***	***VASOPRESSIN***
Gen. effects On Behavior:	PROSOCIALITY Social attention	DEFENSIVENESS Territoriality
Emotions & Patterns of	Relaxation/Recovery "Immobilization without fear"	Vigilance Mobilization & Anxiety
Coping:	PASSIVE	ACTIVE
Effects on Healing:	CHRONIC HEALING	ACUTELY PROTECTIVE
And the Autonomic Nervous syst:	PARASYMPATHETIC Nervous system & SYMPATHETIC N.S	SYMPATHETIC Nervous system

WHAT DOES RESEARCH thus far IN PRAIRIE VOLES TELL US ABOUT THE REGULATION OF OXYTOCIN AND VASOPRESSIN?
Working hypotheses:

FACTORS INCREASING OXYTOCIN RELEASE
- INTENSE STRESSORS (male and female – active coping?)

FACTORS INCREASING OXYTOCIN SYNTHESIS
- CHRONIC STRESSOR (isolation) (female – passive coping?)
- ESTROGEN
- Possibly adversity in early life?

FACTORS INCREASING OXYTOCIN RECEPTOR mRNA
- MILD STRESSOR (1 hr isolation) (female only – coping?)

FACTORS DECREASING OXYTOCIN RECEPTOR mRNA
- CHRONIC STRESSOR (28 days isolation) (male & female)

FACTORS INCREASING VASOPRESSIN SYNTHESIS & AVPR sens.
- ANDROGEN – (Increased territoriality & vigilance?)
- VASOPRESSIN may be particularly important to
  - ACTIVE COPING & MOBILIZATION

SEX DIFFERENCES ARE OFTEN NOT IDENTIFIED IN THE ABSENCE OF STRESSORS.

*Male and female prairie voles do NOT usually differ in the distribution of either oxytocin or vasopressin receptors.

*Also under optimal conditions BASAL OT is typically similar in males and females.

However,…. In the face of challenges or stressors..

MALES AND FEMALES DIFFER in their RESPONSES including to:

* Manipulations of OT/AVP during early life.
* Effects of early experience (handling, etc)
* Chronic stressors, such as isolation
* Are FEMALES protected by more OT Receptors (in N, accumbens) and perhaps the synthesis and release of endogenous oxytocin?? Or is there a sexually-dimorphic epigenetic change in the OXTR? FEMALES may have more OT receptors?
* MALES also may be normally protected by OT and/or AVP, but may be also dependent on and vulnerable to disruptions of AVP, especially in early life. Factors that disrupt AVP may leave males more "anxious," vigilant, and "mobilized, and less capable of managing some kinds of social challenges, that require immobility – also to be continued….

Neonatal exposure to the oxytocin peptide has dose-dependent and sexually-dimorphic behavioral effects.
(Males were in general more sensitive than females to BEHAVIORAL effects of low doses of OT administered POSTNATALLY.)

What happens following experimentally increasing or inhibiting the effects of OXYTOCIN in early life? This can be done by adding synthetic OT (medically "pitocin")

What happens following NEONATAL exposure to an OXYTOCIN RECEPTOR ANTAGONIST (OTA)?

This was addressed by administering neonatal treatments in male and female prairie voles.

On postnatal day 1 animals (male or female)
were treated with

a. no treatment
b. SALINE
c. Oxytocin (OT – low dose)
d. Oxytocin antagonist (OTA) (Manning compound VI)

PEPTIDE RECEPTORS

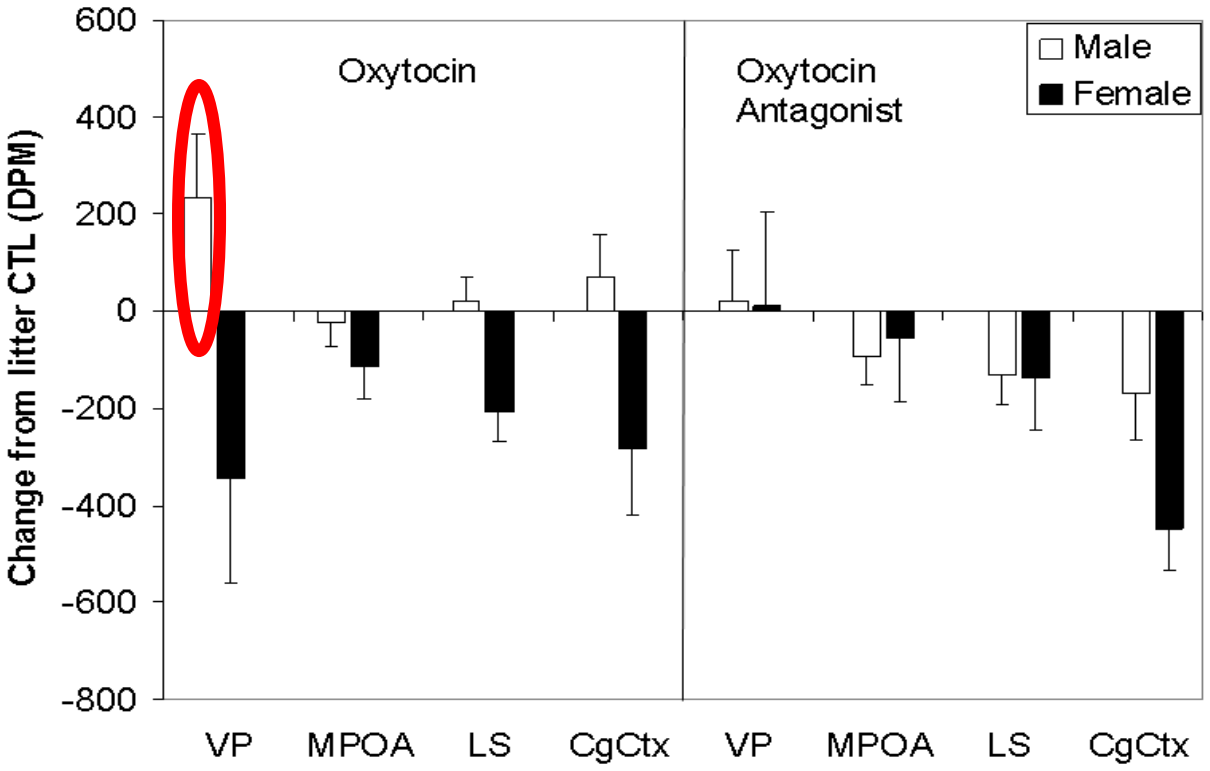
Do early hormonal manipulations including EXOGENOUS OT or an OTA have long-lasting effects on peptides and/or their receptors? YES

The effects were most apparent in:

VASOPRESSIN (V1a) Receptors,

NO significant effects were detected in oxytocin receptors using autoradiography to measure receptor abundance. More sensitive methods might have produced differences??

Changes in Vasopressin V1aR following postnatal OT (3ug) or OTA (0.3ug) were sexually dimorphic and brain region specific.



Bales, et al., Neuroscience, 2007

ENDOGENOUS PEPTIDES

Do early hormonal manipulations including EXOGENOUS OT have long-lasting effects on OXYTOCIN OR VASOPRESSIN PEPTIDES? Yes

VASOPRESSIN PEPTIDE (measured by immunoreactivity) DECREASED in MALES exposed to an OTA.

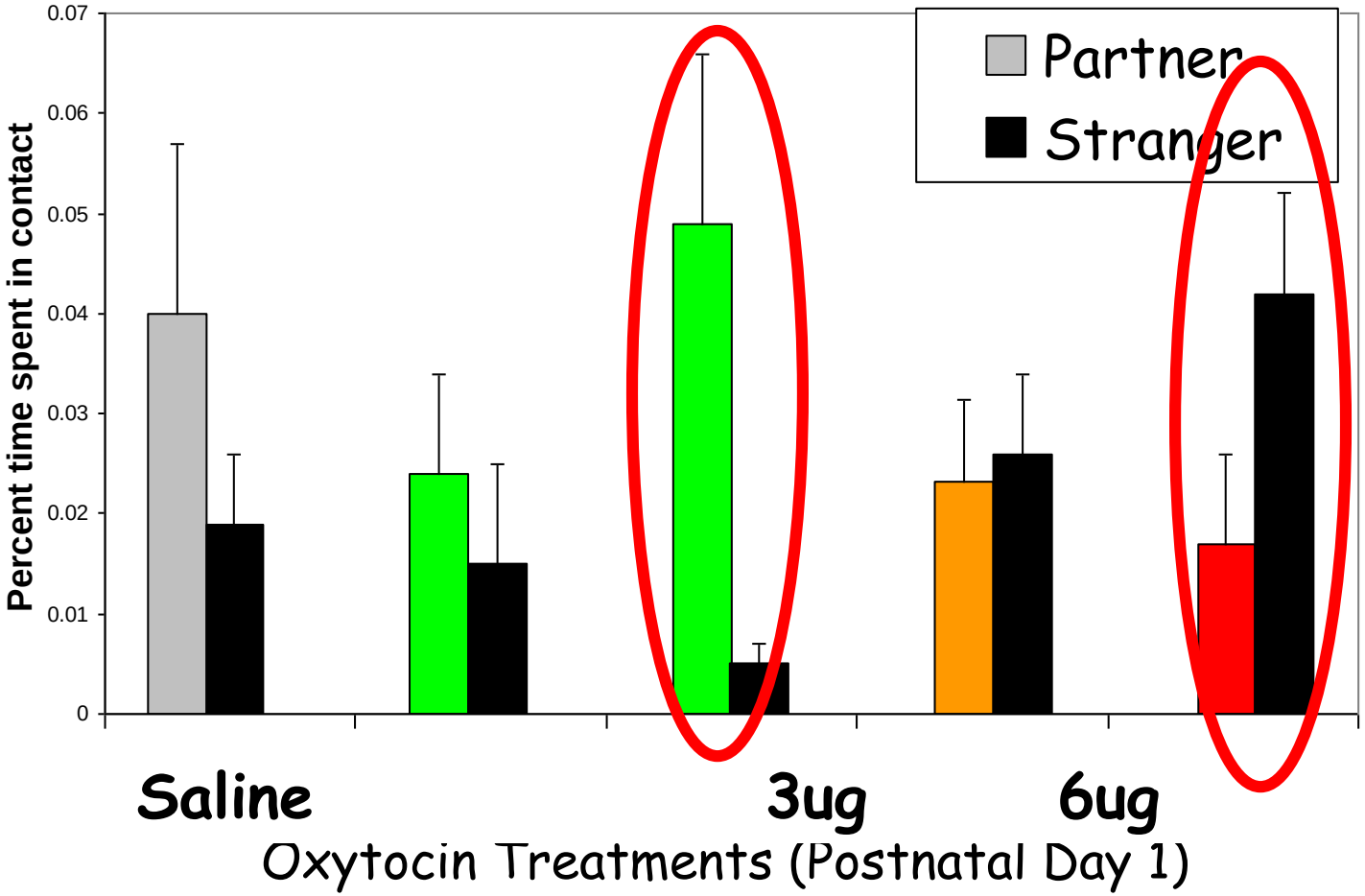
After EXOGENOUS OT or OTA, OXYTOCIN-immunoreactivity in hypothalamus…

Increased Slightly in males, and

Increased Significantly in FEMALES

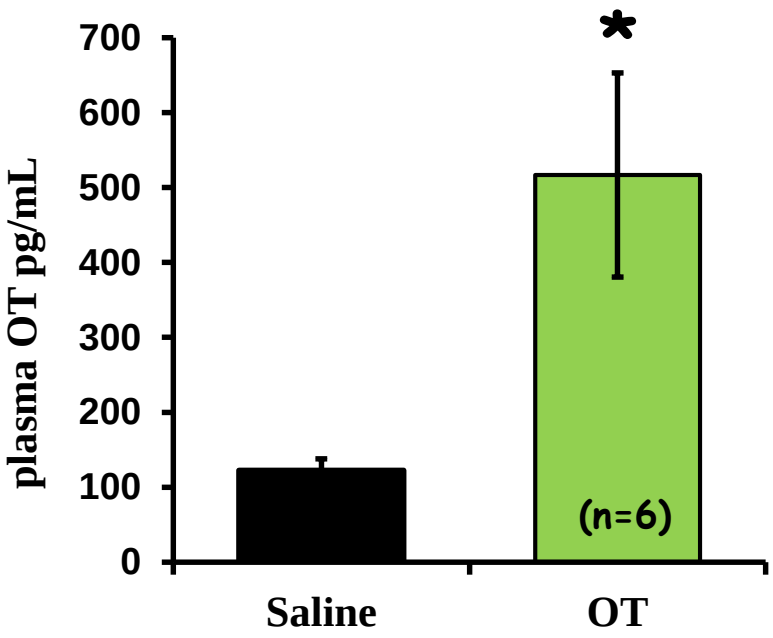
THE BEHAVIORAL EFFECTS OF EXOGENOUS OT (NEONATAL DAY 1) ARE DOSE-DEPENDENT.

WHAT HAPPENS TO PAIR BONDING IN FEMALE PRAIRIE VOLES EXPOSED TO HIGHER DOSES OF NEONATAL OXYTOCIN? Moderate doses of OT facilitated; High doses of OT inhibited preference for the familiar partner (perhaps a partner aversion).



Bales et al., Horm Behav, 2007

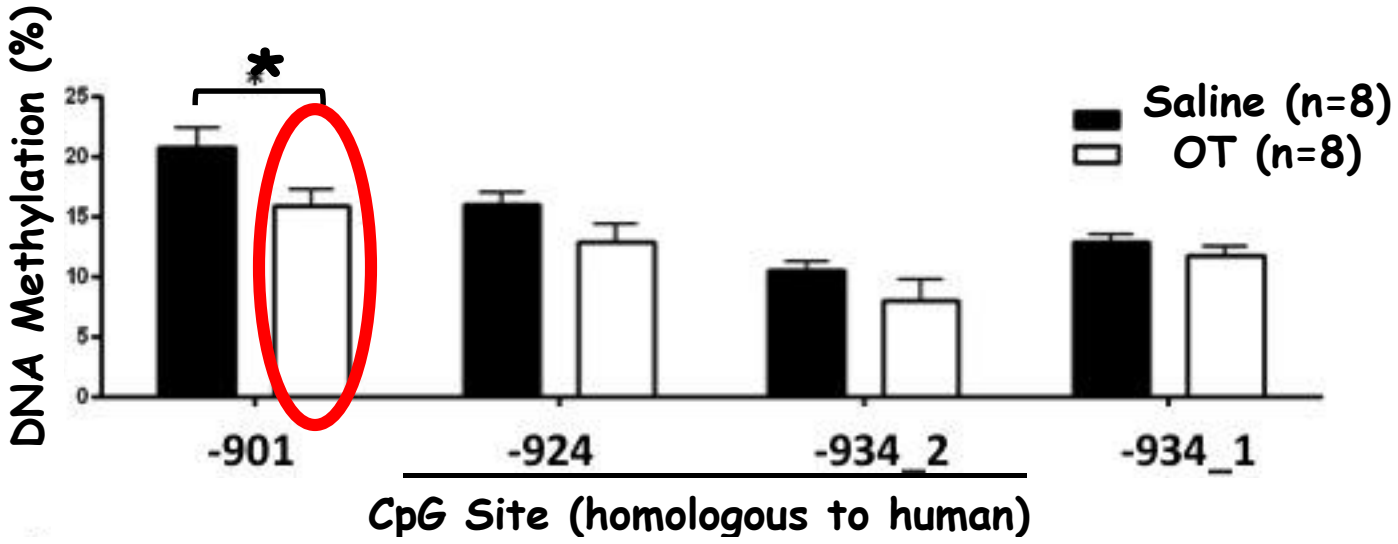
CAN MATERNALLY-INJECTED OT (PITOCIN) REACH THE INFANT?
Yes – at least in prairie voles



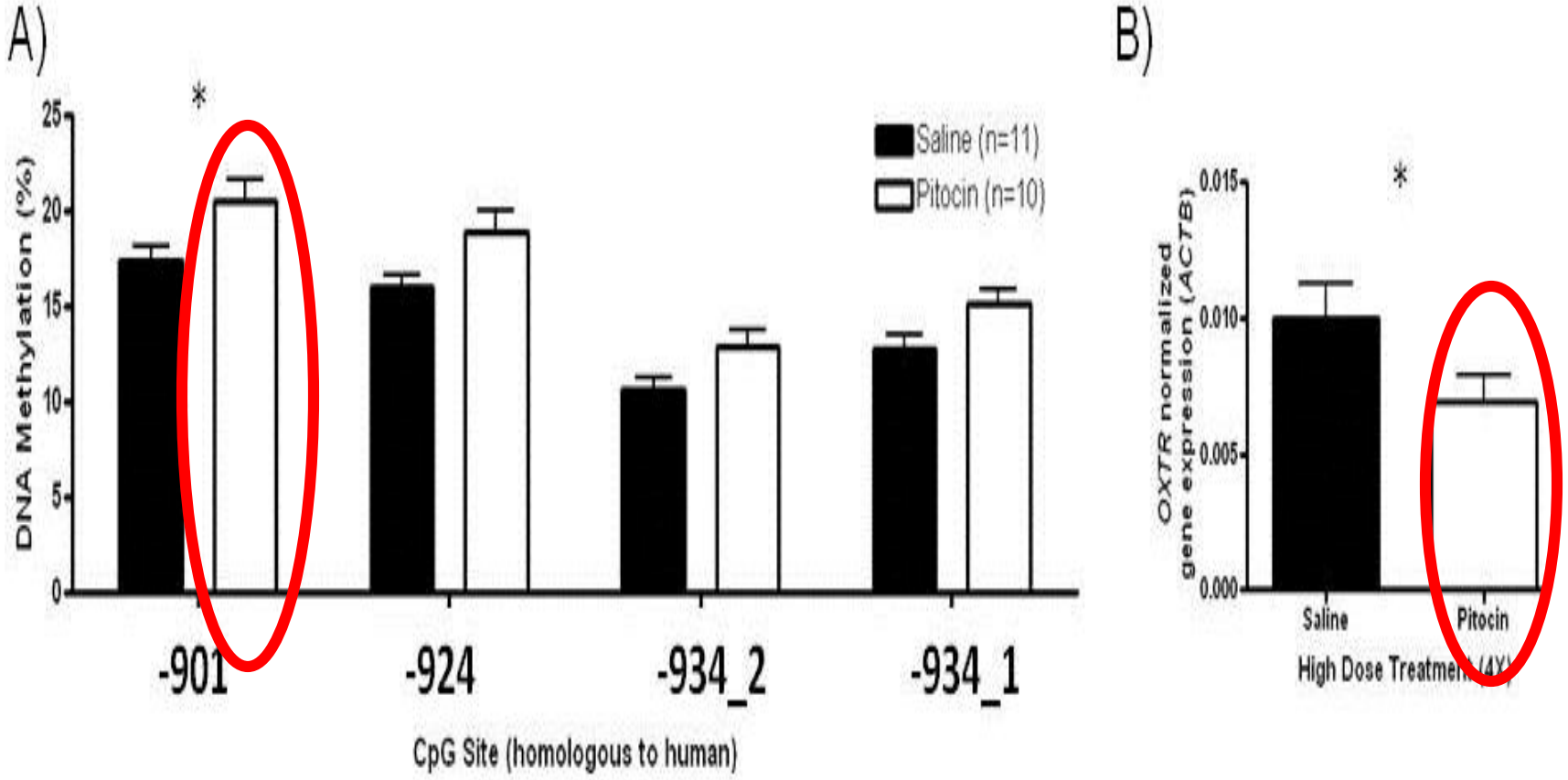
Fetal plasma OT increased following maternal OT treatment

This increase in OTR may be due (at least in part) to DECREASED OTR Promoter Methylation, seen Following Maternal OT Treatment (LABOR AUGMENTATION)

LOW DOSE OF OT (single injection)



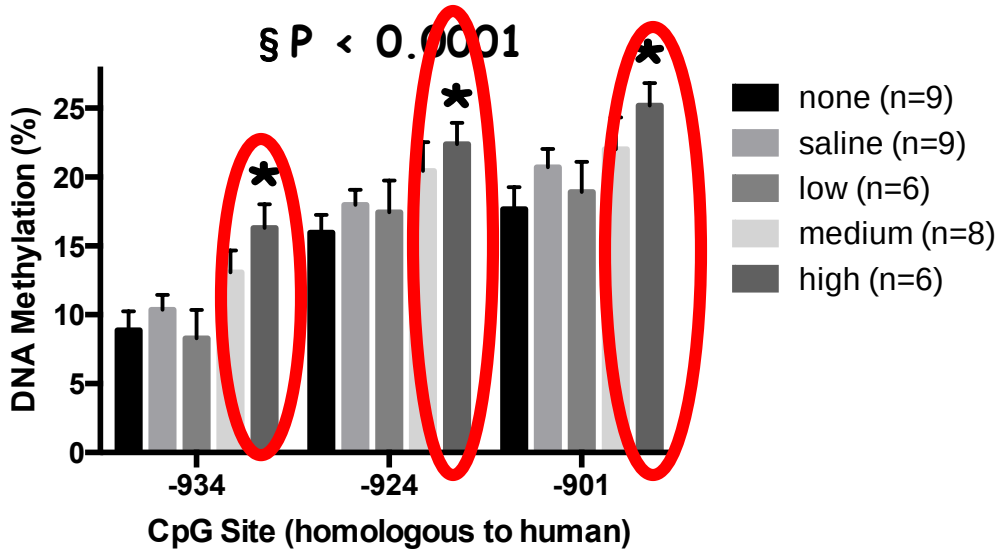
WHEN DOSES OF MATERNAL OT (PITOCIN) WERE HIGH (4 injections), THE EFFECTS WERE THE OPPOSITE: INCREASED METHYLATION OF THE OXTR, AND DECREASED GENE EXPRESSION FOR THE OXTR.



†Two-way ANOVA, main effect of treatment F(1,76)=19.19, P<0.0001;
CpG site F(3,76)=29.29, P<0.0001; no interaction; *significant by Bonferroni posttests, P<0.05

*Mann–Whitney U test (2tailed)
U=4.000, n1=47, n2=19, P=0.05

Oxytocin administered to a term pregnant female (induction of labor model) affects methylation of the oxytocin receptor *in the brains of FEMALE pups*

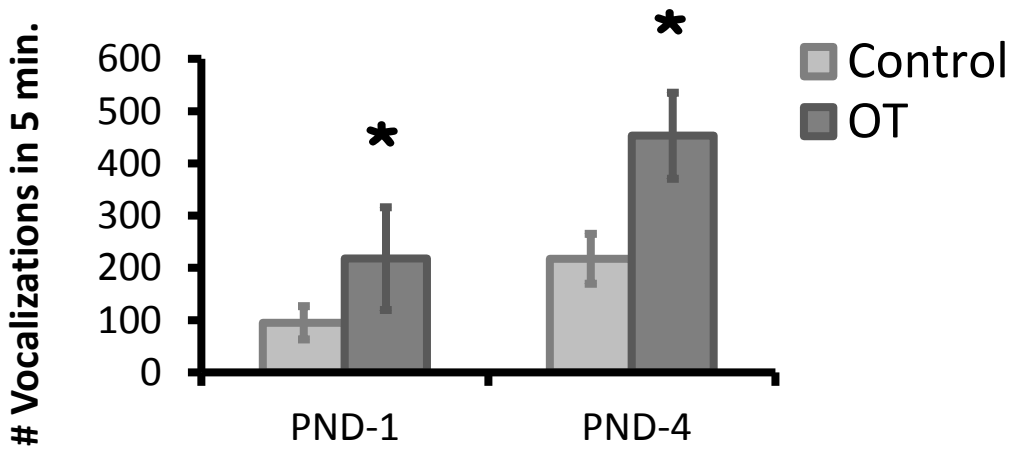


The highest doses of OT (Pitocin) produced a significant increase in DNA methylation of the OXTR. This effect was seen in 3 separate CpG sites. This example is from hindbrain, but the effects were similar in forebrain and midbrain sections

§ Two way ANOVA; *Bonferroni's multiple comparison
OT (high) vs. Saline, p<0.05

A comparable treatment increased vocalizations behavior in infants later in life

Jessica Connelly



Will Kenkel

Hypothesis: (see Carter, C.S. Behavioural Brain Research, 2007 for details):

OXYTOCIN (and OTRs), which are ESTROGEN dependent, may be protective against some of the features of autism -

Possibly accounting in part for the fact that females are less likely than male to exhibit autism/ASD.

VASOPRESSIN, which can be ANDROGEN dependent,

may at high levels, may be associated with vulnerability to ASD -

Possibly contributing to the MALE BIAS in AUTISM (ASD)

PRADER-WILLI and FRAGILE-X symptoms also may be related to relative availability of oxytocin and vasopressin. These may reflect decreases in <u>functional</u> OXYTOCIN and/or INCREASED sensitivity to VASOPRESSIN (or disruptions in vasopressin?)

SEX DIFFERENCES ARE OFTEN NOT IDENTIFIED IN THE ABSENCE OF A CHALLENGE. Why??

*Male and female prairie voles do NOT typically differ in the distribution of either oxytocin or vasopressin RECEPTORS. (exception – Cortex?) Under optimal conditions BASAL OT is typically similar in males and females. Responses to acute stressors may appear similar, however In the face of challenges or stressors (possibly the stress of birth) – MALES AND FEMALES DIFFER in their RESPONSES including to:

* Manipulations of OT/AVP during early life.
* Effects of early experience (handling, nurture, etc)
* Chronic stressors, such as isolation (females inc OT)

MALES – ARE OFTEN MORE SENSITIVE TO THE POSTNATAL ENVIRONMENT

- Evolved differential reproductive success (sperm vs eggs, subordination, etc)
- XY vs XX genetics?

Endogenous steroids

- females – estrogen protective – may facilitate sociality - OT or OTR
- males – androgens increase vulnerability, incl AVP -defense & vigilance?
  - MORE EPIGENETICALLY DEPENDENT ON OT/AVP POSTNATALLY
- **MALES MAY BE ESPECIALLY SENSITIVE TO DISRUPTIVE EVENTS IN THE PERINATAL PERIOD?**
- **MALES ARE SLOWER TO MATURE THAN FEMALES, esp THE PROTECTIVE GABA SYSTEM IN EARLY LIFE?**


BABIES also released
OXYTOCIN in
human fathers (and
grandfathers and
alloparents)

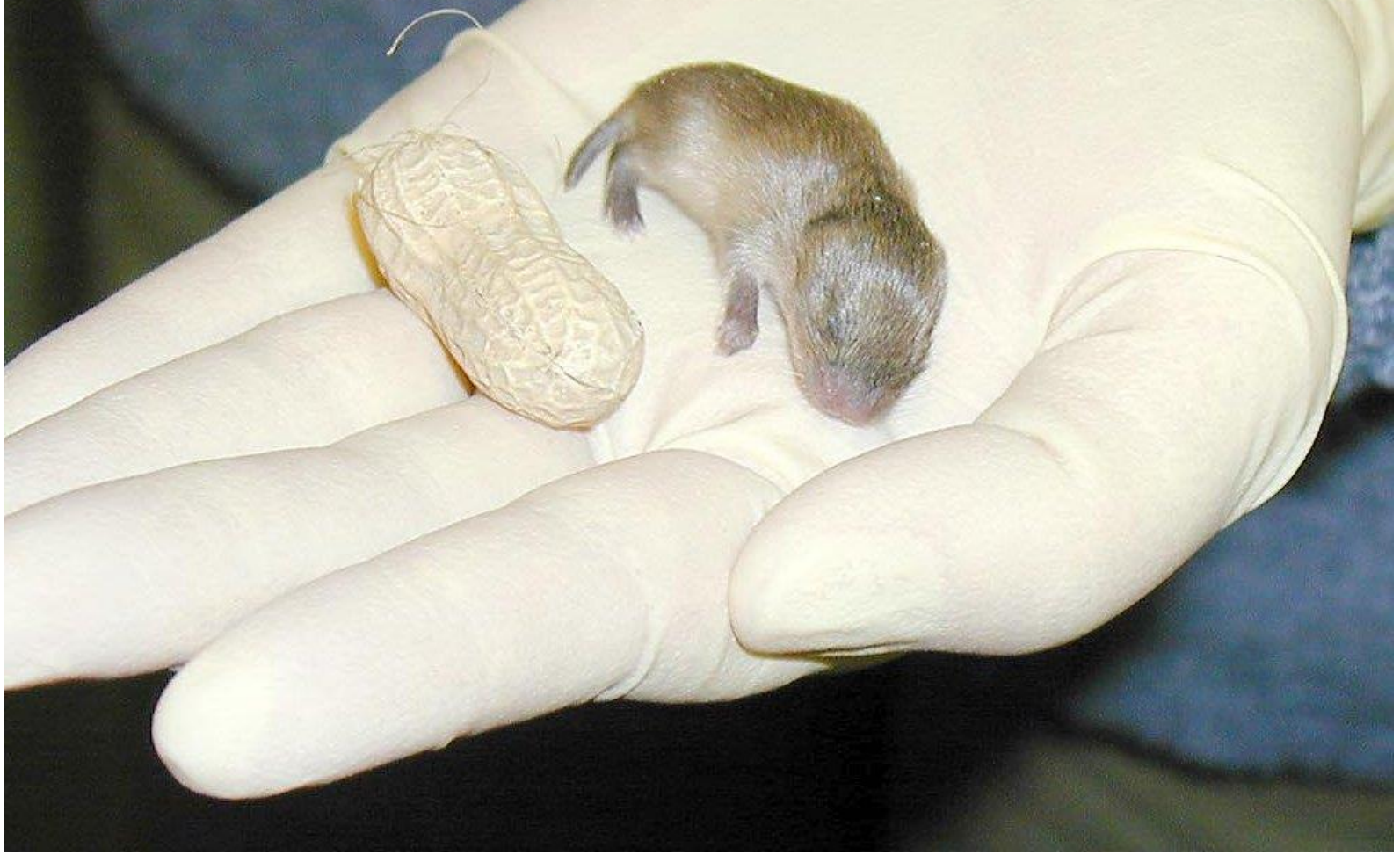

The same hormones that affect mothering, also influence FATHERING –Although in slightly different ways.

Hormones, like OXYTOCIN and VASOPRESSIN, are ADAPTIVE and can protect both father and child.

The consequences of being with a child may benefit both father and child, behaviorally & physically.

FATHERS MAY BE PARTICULARLY PROGRAMMED TO PROTECT THEIR CHILDREN, POSSIBLY IN PART THROUGH THE EFFECTS OF VASOPRESSIN – BUT THIS CAN COME WITH A BIOLOGICAL OR BEHAVIORAL PRICE.

Prairie Voles – When presented with a Pup Most Show Alloparental or Parental Behavior - Including Reproductive Naïve Males (Parenting is does not require learning or experience in this species.)



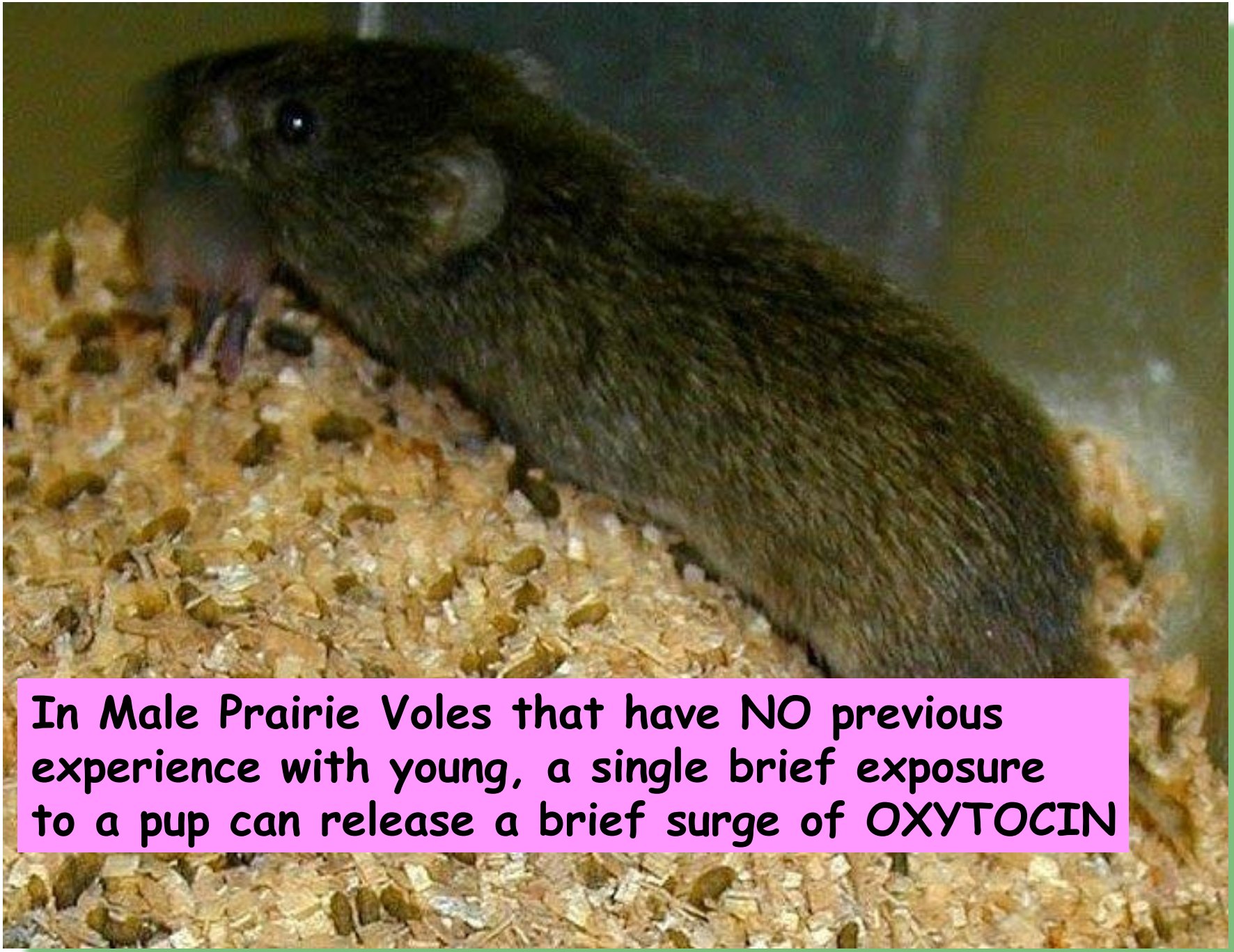
Baby vole –
The size of a peanut but much more behaviorally powerful

Oxytocin facilitates PARENTAL and ALLOPARENTAL BEHAVIOR: Pup retrieval and care by Either the parent or juveniles

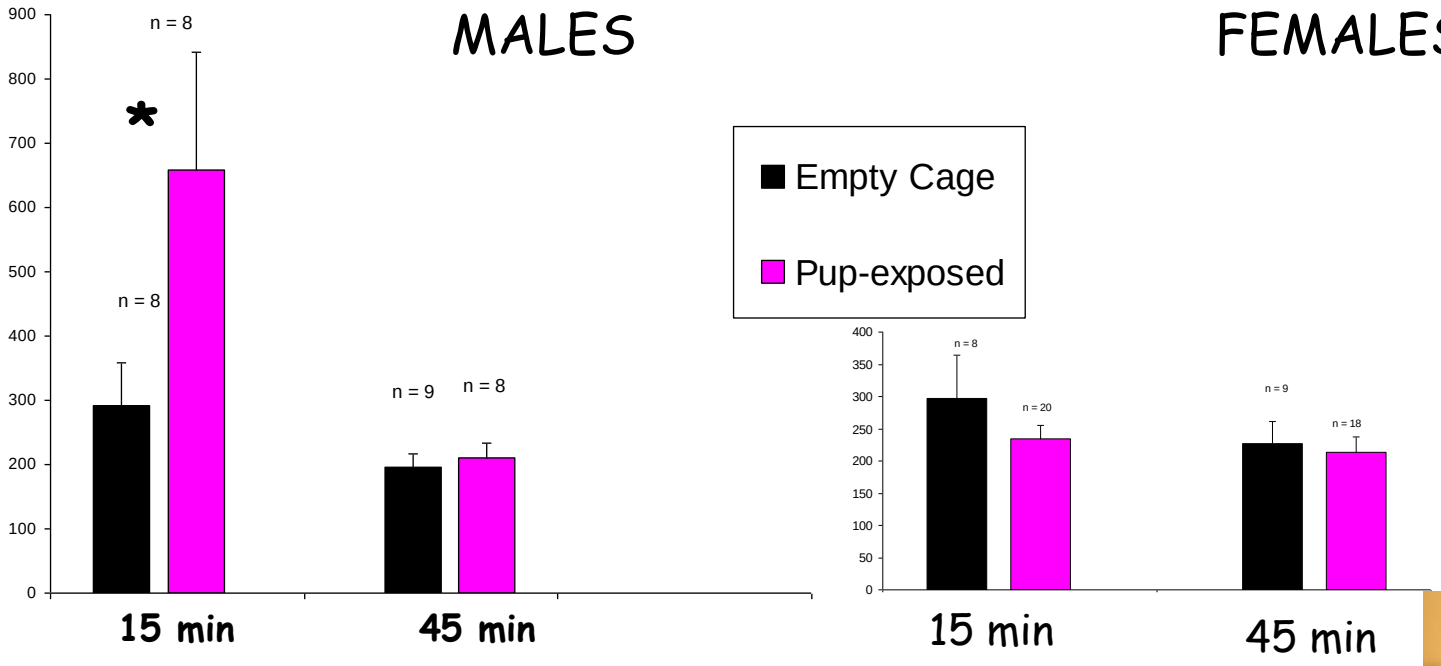
In male prairie voles exposure to an infant has both short-term and long-term consequences.

In Adolescent or Adult Voles as little as:
10-20 min of Exposure to PUP

a. **INCREASED OXYTOCIN (in MALES)**
b. **Reduces Corticosterone and CRH (hpa axis activity)**
c. **Reduced behavioral ANXIETY**
d. **Facilitate subsequent SOCIAL BONDING**
e. **Increased NEUROGENESIS (birth of brain cells)**


In Male Prairie Voles that have NO previous
experience with young, a single brief exposure
to a pup can release a brief surge of OXYTOCIN

Blood levels of OXYTOCIN increased in MALES, but NOT females immediately following 10 min PUP EXPOSURE



Kenkel, Paredes, Yee, Pournajafi-Nazarloo, Bales, & Carter, 2013

Will Kenkel

Exposure to an infant (and other forms of social behaviors as well) can

increase oxytocin

decreases stress hormones (cort) -

and we hypothesized might have

either direct or indirect

effects on NEUROGENESIS

(birth of new neurons - even in the adult brain)

SHOULD OXYTOCIN BE A MEDICINE??

Oxytocin is already available on the internet as an intranasal spray. It is NOT a controlled substance. It has NOT been evaluated by the FDA. We have NO information about the CHRONIC effects of exogenous oxytocin. (Chronic elevations in OT were associated with a down-regulation of OT receptors… Will this happen if OT is chronically used as a "medicine?" Probably!!)

However, used wisely it may have a role in medicine. Several drug companies are testing OT-based compounds for the treatment of autism, schizophrenia and other disorders.

Oxytocin (Pitocin) is routinely used during birth and in the postpartum period. We know essentially NOTHING about the consequences for the child or mother of these treatments.

OXYTOCIN IS ONE COMPONENT OF A COMPLEX NEUROENDOCRINE-AUTONOMIC SYSTEM. We must have a deeper knowledge of the natural regulation of this system, especially in early life.

Knowledge of ENDOGENOUS oxytocin may serve as a metaphorical Rosetta Stone for understanding natural healing.

I suggest we start there.