DIRECT CONNECTIONS BETWEEN THE BRAIN AND THE AREA OF THE SPINAL GENERATOR FOR EJACULATION:

A NEUROANATOMICAL TRACING STUDY IN THE RAT

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RATIONALE

- Lumbar spinothalamic (LSt) neurons identified in rats as spinal generator for ejaculation (SGE) in L2-L4 spinal segments (Truitt & Coolen, 2002)
- LSt project to thalamus (parvicellular subparafascicular nucleus) (Ju et al., 1987)
- LSt project to spinal ejaculatory centres (S, PS, and somatic)
- Brain descending projections to LSt still not described
- LSt under activatory/inhibitory influences from brain

Chen X. et al Neuroscience 2005, 2006; Sun XQ et al Neuroscience 2009

OBJECTIVE

- To identify brain structures projecting onto LSt by injecting retrograde tracer into LSt area
brain areas

excitatory/inhibitory control

spinal cord

DGCiML
Lst
LSC
MG
PN
PdN
SPN
DM

IMG
HN

vas deferens
prostate
seminal vesicle

BS muscle

meatus

bladder neck

S1-L6 L6-L5
L1-T13
Retrograde tracing technique for detecting neural connections from synapse in SGE to soma in brain.
MATERIALS AND METHODS

- Wistar male rats sexually naive (250-300 g)
- Partial laminectomy between T13-L1 vertebrae
- Glass capillary lowered in spinal cord (L3-L4)
- Injection of 2% fluorogold (0.2 µl) using hydraulic microdriving system
- Capillary removed 5 min after injection end + agar in laminectomy
- Animals kept over 14 days and then perfused (PBS + PAF4%)
- Spinal cord and brain collected and sliced with cryostat (40 µm thick)
INJECTION SITE OF THE DYE IN THE SPINAL CORD

Caudo-rostral spreading: L4-L2

Intense fluorogold signal in areas X and VII medial

Moderate fluorogold signal in areas VI and VIII

Spinal cord sections

Cresyl violet

Fluorogold signal
RESULTS: FLUOROGOLD-POSITIVE BRAIN SITES

- MEDULLA OBLONGATA
  - GiV
  - Gi

- DPGi: dorsal paragigantocellular
- Gi: gigantocellular
- GiA: gigantocellular alpha
- GiV: gigantocellular ventral
- MdV: medullary reticular, ventral
- MVe: medial vestibular
- RMg: raphe magnus
- Rob: raphe obscurus
- RPa: raphe pallidus
RESULTS: FLUOROGOLD-POSITIVE BRAIN SITES cont’d

LH: lateral hypothalamus
Pr5: sensory trigeminal nucleus
Red: red nucleus
RMg: raphe magnus
S1HL: primary somatosensory cortex, hindlimb region
# RESULTS: QUANTITATIVE DATA

<table>
<thead>
<tr>
<th>MEDULLA OBLONGATA</th>
<th>PONS</th>
<th>FOREBRAIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cell Nb / mm²</td>
<td>Mean cell Nb / mm²</td>
<td>Mean cell Nb / mm²</td>
</tr>
<tr>
<td>DPGi</td>
<td>MdV</td>
<td>LVe</td>
</tr>
<tr>
<td>5.1 ± 1.1</td>
<td>5.6 ± 2.2</td>
<td>10.4 ± 5</td>
</tr>
<tr>
<td>Gi</td>
<td>RMg</td>
<td>MVe</td>
</tr>
<tr>
<td>4.9 ± 0.6</td>
<td>7.1 ± 1.6</td>
<td>5 ± 2.3</td>
</tr>
<tr>
<td>GiA</td>
<td>ROb</td>
<td>Pr5</td>
</tr>
<tr>
<td>13.1 ± 3.6</td>
<td>12.6 ± 1.5</td>
<td>4.5 ± 3.1</td>
</tr>
<tr>
<td>GiV</td>
<td>RPa</td>
<td>Red</td>
</tr>
<tr>
<td>40.2 ± 7.4</td>
<td>31.7 ± 1.5</td>
<td>17.3 ± 11.3</td>
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<tr>
<td>LPGi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.8 ± 3.3</td>
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</tbody>
</table>

Data expressed for each structure as mean number of cells per mm² in 3 rats.
CONCLUSION

• 15 brain structures found with direct projections onto LSt cells /SGE area

• Highest density of projecting neurons found in the gigantocellular ventral (GiV) and raphe pallidus (Rpa)

• Among the FG-positive brain structures gigantocellular (Gi), gigantocellular alpha (GiA), GiV, LPGi, and RPa are known to be involved in ejaculation

• This study points out brain structures to be targeted for identifying the exact neuronal population projecting to LSt/SGE

• Next step: anterograde tracer in Gi subnuclei and RPa coupled with immunodetection of LSt