The Pedunculopontine Nucleus (PPN) in Parkinson’s Disease

Anatomical Organization of the Pedunculopontine Nucleus: Pathology in Parkinson’s Disease

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Relevance of the PPN in PD

- PPN is a significant source of inputs to all basal ganglia nuclei (particularly massive to SNc and STN).
- PPN receives massive GABAergic inputs from basal ganglia output nuclei (GPi, SNr) and from STN.
- Cholinergic neurons in PPN undergo massive degeneration in Parkinson’s disease.
- Bilateral lesion of cholinergic cells in PPN induces gait problems in monkeys.
- Deep brain stimulation in the “area of the PPN” reduces gait and balance problem in “some” Parkinson’s disease patients.
- The PPN is involved in numerous motor and non-motor functions that are affected in PD (sleep, gait, balance, eye movements, state of vigilance etc.).
Where is the PPN located??

- Upper brainstem (from caudal midbrain to upper pons). Wraps around axons of the superior cerebellar peduncle.
- Made up of two major parts
  - **PPNc (compacta):** Caudalmost part, contains the majority of cholinergic neurons.
  - **PPNd (diffusa):** Rostralmost part, contains mainly GABAergic and glutamatergic neurons. The main recipient of BG projections.
- **Cuneiform Nucleus (CN):** Dorsal to PPN. Lateral CN and PPN are part of the Mesencephalic Locomotor Region (MLR). May also control gait, posture??

[Fournier-Gosselin et al., 2013, Mov. Dis. 28: 1330]
PPN is located in the vicinity of major axonal tracts

- **CTT**: Rubro-olivary fibers, taste inputs to thalamus
- **LL**: Lateral lemniscus (auditory inputs)
- **ML**: Medial lemniscus (somatosensory inputs)
- **SCP**: Superior cerebellar peduncle (Cerebellar outflow)
- **STT**: Spinothalamic tract (pain and temperature inputs)

[Fournier-Gosselin et al., 2013, Mov. Dis. 28: 1330]
The PPN is a major source of acetylcholine to forebrain and brainstem structures

The PPN and the laterodorsal tegmental (LDT) are the Ch5 (PPN) and Ch6 (LDT) cholinergic cell groups in the mammalian CNS

Mesulam et al., 1983, Neuroscience 10:1185
Mesulam et al., 1989, JCN 281: 611
The PPN also comprises a large population of GABAergic neurons
(Mena-Segovia et al., 2009, JCN 515: 397)

GAD and ChAT-containing neurons are partly segregated along the rostrocaudal extent of the PPN. GAD-containing cells are more anterior than cholinergic neurons
Cholinergic, GABAergic and glutamatergic neurons form three distinct populations of cells in the rat PPN (Wang and Morales, 2009, EJNS 29:340)

Overall, GABAergic and glutamatergic neurons are more abundant than cholinergic cells in PPN.
- Cholinergic and glutamatergic cells are abundant in PPNc, but also present in PPNd.
- GABAergic cells predominate in PPNd.
- Less than 5% PPN neurons co-express cholinergic and non-cholinergic markers.

### Table 3. Sizes of three distinct subpopulations of neurons in the PPTg:

<table>
<thead>
<tr>
<th>Neurons expressing</th>
<th>%</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
<th>n</th>
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<tbody>
<tr>
<td></td>
<td>ChAT</td>
<td>vGluT2 mRNA</td>
<td>GAD mRNA</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PPTg area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pars compacta (n = 2093)</td>
<td>31 ± 3</td>
<td>652</td>
<td>50 ± 4</td>
<td>912</td>
<td>19 ± 2</td>
<td>372</td>
</tr>
<tr>
<td>Pars dissipata (n = 3789)</td>
<td>23 ± 3</td>
<td>760</td>
<td>37 ± 2</td>
<td>1392</td>
<td>40 ± 4</td>
<td>1637</td>
</tr>
<tr>
<td>Totals in PPTg (n = 5725)</td>
<td>27 ± 2</td>
<td>1412</td>
<td>43 ± 2</td>
<td>2304</td>
<td>31 ± 3</td>
<td>2009</td>
</tr>
</tbody>
</table>
Other studies have shown evidence for co-localization of glutamate or GABA with ChAT in primate and non-primate PPN (Lavoie and Parent, 1994, JCN 344: 190)
Other chemical markers in PPN
(Vincent, 2000, J. Chem Neuroanat. 18:23)

- **NADPH-diaphorase (Nitric Oxide-NO):** Complete co-localization with ChAT. Often used as marker of cholinergic cells in PPN.

- **Calcium binding proteins** (calretinin, parvalbumin, calbindin) - Partly co-localize with ChAT.

- **Neuropeptides** (Substance P, CRF, bombesin/gastrin-releasing peptide, atriopeptides) - Partial co-localization with ChAT.
Afferent Connections to the PPN

**BASAL GANGLIA NUCLEI**
- Globus Pallidus, internal segment
  - Ventral pallidum
  - Substantia nigra reticulata
  - Subthalamic nucleus

**CEREBRAL CORTEX**
- Motor cortices
- Primary auditory cortex
- Medial Prefrontal cortex

**BRAINSTEM**
- Raphe, locus coeruleus
  - Superior colliculus
  - Contralateral PPN
  - Mesopontine rostromedial tegmental nucleus

**POSSIBLE AFFERENTS**
- Dorsal striatum + accumbens
  - Zona incerta, Habenula
  - Cerebellum
  - Spinal cord
GPI outflow to PPN and thalamus are collaterals of single pallidal axons
GPI and SNr Projections to PPN Avoid Cholinergic Cells
(Shink et al., 1997, JCN 382: 348)

- PHA-L injections in various functional territories of monkey GPI.
- Most GPI terminals are located in PPNd and do not establish close interactions with cholinergic cells.
- Same pattern was found for SNr projections to PPN in rats.
Efferent Connections of the PPN

**BASAL GANGLIA (Chol. + non-chol.)**
- Dopaminergic SNc and VTA
  - Subthalamic nucleus
    - Globus pallidus
    - Striatum

**THALAMUS (mainly chol.)**
- Intralaminar nuclei
- Sensory relay and ventral motor nuclei
- Associative high order nuclei
  - Reticular nucleus

**BRAINSTEM/SPINAL CORD**
- Reticular formation (Chol.)
  - Nucleus Pontis oralis
  - Gigantocellular nucleus
  - Cervical and thoracic sp. Cord (non-chol.)

**OTHER EFFERENTS**
- Nucleus basalis of Meynert
- Inferior and superior colliculi
  - Hypothalamus
PPN Projections to SNc and STN are more massive than inputs to other basal ganglia nuclei.

Anterograde labeling in SNc and STN after radioactive amino acid injections in the monkey PPN.

Lavoie and Parent, 1994, JCN 344:210
Descending Projections of PPN

- Mainly studied in rodents and cats. Very few primate studies.
- Have direct access to pontine and medullary nuclei that project to the spinal cord (Rye et al., 1988, JCN 269: 315; Grofova and Keane, 1991, Anat Embryol 184:275)
- In rats, cholinergic neurons contribute to the PPN projections to medullary and pontine reticular formation (Rye et al., 1988, JCN 269: 315). Some of these neurons send axon collaterals to the thalamus (Semba et al., 1990, Neuroscience, 38:643).
- In rats, PPN projections to cervical spinal cord originate mainly from non-cholinergic cells (most likely those that receive major basal ganglia inputs) (Rye et al., 1988, JCN 269: 315).
- Cholinergic neurons in PPN are part of the Mesencephalic Locomotor Region (MLR) (Garcia-Rill et al., 2014, J. Neural Transm).
- Involved in REM sleep (initiation??ataonia??).
The extent of PPN cholinergic cell loss is more prominent in faller than in non-faller PD patients.
Food for Thoughts???

- Which populations of PPN neurons are affected by basal ganglia outputs??
- Is the PPN a relay for basal ganglia output to gain access to the reticulospinal systems ??? spinal cord??
- Is there significant cross-talk between cholinergic, GABAergic and glutamatergic neurons in PPNc and PPNd??
- Do the descending projections of PPN control gait and posture?? Do they have anything to do with voluntary limb movements??
- How does basal ganglia outflow to the thalamus and PPN (collaterals of the same axons) control voluntary movements??
- Is the degeneration of cholinergic neurons in PPN responsible for gait problems in PD? How?? What networks are affected?? How does DBS relieve some of these problems??
Pedunculopontine Nucleus and Parkinsonism

Thomas Wichmann, MD
The PPN Interacts with the Basal Ganglia

Characteristics of Human PPN  
(Weinberger et al. (2008))

- 5 PD and 2 PSP patients,
- Cellular characteristics within PPN marginally different from those of cells above and below it
- 38% of neurons in the PPN responded to active or passive ‘movements’. 27% of neurons above it, and 33% of neurons below it responded similarly
- Also describe paresthesias and eye movements upon microstimulation above, within and below the PPN
- Oscillatory activity:
  - 35-50 Hz LFP oscillations ‘sometimes observed’ within or below PPN
  - No oscillatory activity in single cell recordings, no coherence between single cell activity and LFP signals

Characteristics of Human PPN
(Shimamoto et al. (2010))

• 2 patients with PD, 2 with atypical parkinsonism
• Cells in PPN were more bursty, less oscillatory and had a lower firing rate than cells dorsal to it
• Responses to passive movement and eye opening, dorsal to, ventral to, and within PPN
• Describe LFP recordings in one patient above and within PPN
  – LFP spectrum changes with movement

Shimamoto et al. (2010) JNPP 81: 80
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Characteristics of Human PPN
(Tattersall et al. (2014))

- PPN neuron recording in 11 patients (10 with PD)
- PPN neurons discharge as functional networks whose activity is phase-locked to alpha oscillations
- PPN neurons respond to limb movement and imagined gait
  - different networks are activated during imagined gait and movement

Tattersall et al. (2014) Nat Neurosci 17: 449
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Effects of Parkinsonism on PPN Activity

• Lesions of the nigrostriatal tract increase PPN firing

Effects of Parkinsonism on PPN Activity

- Lesions of the nigrostriatal tract increase PPN firing
- Increased coherence between cortical and PPN LFPs, with reduced lag between motor cortex and PPN

Valencia et al. (2014) J Neurophysiol 111: 434
Effects of PPN Manipulations on STN, SNr, VL Activity in Parkinsonian Rats

- Breit et al. (2001, 2006): PPN lesions reduce STN and SNr firing rates (and normalize firing patterns)
- Galati et al. (2008), Park et al. (2014): PPN stimulation (25 Hz) reduces firing in STN and SNr, particularly in neurons with burst and random patterns
- Liu et al. (2011): PPN stimulation (low frequency) increases firing in the VL thalamus in normal or parkinsonian rats.
  - Effect blocked by local atropine


Park et al. (2014) Neurosci Lett 577: 16
Effects of PPN Manipulations on SNc Activity in Normal Rats

- Floresco et al. (2003): Electrical stimulation in PPN induces bursts of spikes in SNc
- Okada et al. (2009/2013): Reward-related PPN neurons project to medial and central SNc
- Sensorimotor/arousal/alerting-related PPN neurons project to lateral SNc

Hong and Hikosaka (2014) Neuroscience 282C: 139
Effects of STN Manipulations on PPN in Parkinsonian Individuals

- Breit et al. (2001): STN lesions reduce excessive PPN firing in parkinsonism
- Neagu et al. (2013): STN stimulation produces short- and long-latency latency evoked potentials in the human PPN

Behavioral Effects of PPN Lesions in Monkeys

• Kojima et al. (1997), Aziz and Stein (1997), Aziz (1999): PPN lesions cause flexed posture, arm and leg hypokinesia
  – Animals recover with unilateral, but not bilateral lesions

• Grabli et al. (2013): PPN lesion + MPTP lesion = parkinsonism + gait and balance problems
  – PPN lesion may improve hypokinesia
Behavioral Effects of Temporary PPN Interventions in Normal and Parkinsonian Monkeys (n=2)

Nandi et al. (2002) Brain 125: 2418
More Behavioral Effects of Temporary PPN Interventions in Parkinsonian Monkey (n = 1)

![Bar chart showing movement counts per hour](chart.png)

- Pre-MPTP: n=20
- Untreated post-MPTP: n=29
- L-DOPA: n=15
- PPN stimulation: n=38
- L-DOPA & stimulation: n=17

F(4, 114) = 26.8, P < 0.001

Jenkinson et al. (2006) Neuroreport 17: 639
Summary

- PPN neurons show movement-related responses, reward-related responses
- PPN cells change firing rates and patterns in the parkinsonian state, become more entrained to motor cortex
- Spectral changes in LFP signals very variable
- PPN lesions in normal monkeys
  - reduce STN and SNr firing
  - cause poverty of movement in primates
- PPN lesions in parkinsonian monkeys
  - May have antiparkinsonian effects, but add gait disability
- PPN stimulation/activation
  - Alters STN and SNr firing, depending on firing pattern
  - Improves akinesia/bradykinesia in monkeys

Caveats

• General
  – PPN poorly defined in all available functional studies
  – Recordings of LFPs questionable in this area because of multiple neuronal contributors to LFPs
• Animal studies
  – Rodent recording studies done under anesthesia
  – Primate studies have utilized very few animals
  – Relationship of PPN-inactivation induced akinesia and parkinsonism not clear
• Human recording studies
  – Very few patients, contradictory results
  – Selected and heterogeneous patient populations

PPN DBS for PD

Mahlon DeLong MD
W.P. Timmie Professor of Neurology
The Basal Ganglia

Surgical Targets

- GPe: External pallidum
- GPi: Internal pallidum
- SNr/c: Substantia nigra pars reticulata
- SNc: Substantia nigra pars compacta
- STN: Subthalamic nucleus

The diagram shows a brain with surgical targets labeled.
Proposed Basal Ganglia “Circuit Disorders”

• **Movement Disorders**
  – Parkinson’s Disease
  – Dystonia
  – Hemiballismus
  – Chorea

• **Neuropsychiatric Disorders**
  – Tourette’s Syndrome
  – Obsessive Compulsive Disorder
  – Drug addiction
  – Depression?
Basal Ganglia-Thalamocortical Circuits

Associated Clinical Disorders

**Motor**
- Cortex
  - SMA, PMC
  - CMA, M1
  - Putamen
  - SNr/GPi (motor territory)
  - VLo, VLm, VApC
  - PD, HD, TS, DYSTONIA

**Oculomotor**
- Striatum
  - FEF
  - SEF
  - Caudate
  - SNr/GPi (oculom. territory)
  - VApC
  - PD, HD

**Prefrontal**
- Pallidum
  - DLPFC
  - LOFC
  - Caudate
  - SNr/GPi (assoc. territory)
  - VApC, VAmC
  - PD, HD

**Limbic**
- Subst. nigra
  - MOFC
  - ACA
  - Caudate (ventr.)
  - VS
  - SNr/GPi (limbic territory)
  - VAmC, VLM
  - PD, HD
  - TS, OCD, ADDICTION DEPRESSION

Wichmann and Delong, Neuron (2006)
**Rationale for DBS**

- A technique for selectively removing or altering abnormal network properties which disrupt downstream mechanisms and restoring function
- A major advantage of DBS is that it is highly targeted and focal
  - uncomplicated by remote side effects of pharmacologic agents
- Side effects are minimal
- The feature of adjustability provides the opportunity for repeated fine-tuning and long term benefits
- Reversibility provides the possibility of future treatments requiring an intact basal ganglia thalamocortical circuitry.
DEEP BRAIN STIMULATION (DBS)

A minimally invasive form of neuromodulation with the advantages of a *highly focal intervention* with adjustability of stimulation parameters:

- Amplitude (1-5V)
- Frequency (60-180Hz)
- Pulse duration (60-200microsec)
STN DBS

Paresthesia threshold = 5 V
Dysarthria threshold = 6 V

5 mm
Parkinson’s Disease
*Medical and Surgical Treatments*
*Targeting the Motor Circuit*

**DA replacement**
- DA receptor agonists
- L-DOPA
- DA cell transplantation*
- Spheramine*

**DA extenders**
- MAO B inhibitors
- COMT inhibitors

**Anticholinergics**

**Trophic factors**
- GDNF

**Gene therapy***
- Subthalamotomy
- STN DBS

**Glutamate antagonists**

**CORTEX**

**PUTAMEN**

**GPe**

**STN**

**GPi/SNr**

**Brain stem/Spinal cord**

**PPN**

**CM**

**VL**

**Thalamotomy VL DBS**

**Pallidotomy GPI DBS**

**PPN DBS**
Parkinson’s Disease

Medical and Surgical Treatments
Targeting the Motor Circuit

DA replacement
- DA receptor agonists
- L-DOPA
- DA cell transplantation*
- Spheramine*

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Anticholinergics

Trophic factors
- GDNF

Gene therapy*
- Subthalamotomy
- DBS

- tremor, rigidity
- akinesia/bradykinesia

Brain stem
Spinal cord

PUTAMEN

CM | VL

CORTEX

STN

GPe

GPi

PPN

Thalamotomy
DBS
VLp
tremor
VLa
dyskinesias

Pallidotomy
DBS
tremor, rigidity,
akinesia/bradykinesia

DBS

falls, FOG, instability
akinesia/bradykinesia?

DBS

dyskinesias
Thalamotomy

*Its Place in Functional Surgery*

- Targeting the *cerebellar* receiving area (VLp) is highly effective for *tremor*, but has little or no effect on movement or akinesia/bradykinesia in PD.

- Targeting the *pallidal receiving area* (VLa) is also ineffective for akinesia/bradykinesia but effective for *dystonia, chorea/ballismus and drug-induced dyskinesias*
  - Thalamotomy including both basal ganglia and cerebellar recipient zones in the *normal non-human primate* is *without overt motor deficit*
Akinesia/Bradykinesia
Pallidothalamic vs Pallido-PPN Projections

• The lack of effect of combined lesioning of pallidal (Vla) and cerebellar (VLp) receiving areas of the thalamus on akinesia and bradykinesia in PD and on movement in normal animals strongly suggests a major role for pallidal projections to the brainstem in the pathophysiology of akinesia and bradykinesia, especially, those to the PPN.
PD Motor Complications

• After some time patients with PD may develop motor complications that are poorly or non-responsive to Levodopa
  – loss of postural reflexes
  – Falls
  – Freezing of gait (FOG)

• Falls, especially with hip fractures are a major factor in morbidity and mortality in PD
PPN
*Role in Disorders of Gait and Balance*

- The PP is a portion of the physiologically defined "mesencephalic locomotor region." (Garcia-Rill, 2014)
- Several studies have shown that the cholinergic neurons of the PPN degenerate in PD (Hirsch et al., 1987; Zweig et al., 1987, Jellinger K 1988)) and are strongly implicated in the development of gait and balance disorders
- Stimulation of the PPN can alleviate gait disturbances and falls in advanced PD patients (Stefani et al., 2007; Ferraye et al., 2010; Moro et al., 2010, Mazzoni et al. 2011)
PPN DBS

• There is a clear unmet need to develop novel therapeutic strategies to treat disabling levodopa and DBS resistant axial disturbances in PD.
• Because the PPN is an integral component of the MLR there has been recent exploration of this novel target.
• Supporting data in animal studies suggested that the PPN could be a viable DBS target for treating axial symptoms.
• There have been a number of case reports and small uni- and bilateral non-randomized DBS studies examining short-term outcomes.
PPN DBS

• The initial enthusiasm for PPN DBS has been somewhat tempered by mixed clinical outcomes in subsequent studies, although some patients exhibit clear benefit for axial symptoms.
• Almost all of the studies have been open label and have not had rigidly defined inclusion criteria.
• Standardized outcome measures of postural instability freezing of gait and falls are needed.
• The variability in outcomes may be due to the mix of clinical patient types, differences in surgical approach, lead location, stimulation parameters and the shortcomings of clinical rating scales.
Future Needs

• Unresolved questions:
  – targeting
    • Optimal target? PPNc or PPNd, Cuneate
    • Use of imaging such as diffusion tractography?
    • Role of microelectrode recording
    • Bilateral versus unilateral targeting of PPN?
    • Simultaneous or staged bilateral targeting
    • PPN targeting as an adjunct to STN, GPi or SNr DBS?
  – Programming
    • Most often at 25-50Hz
    • The Toronto group found most benefit at 50–70Hz
      – (other parameters (pulse width and voltage) did not differ)
    • PPN also modulates REM sleep, cognition, mood, attention, arousal-caution/benefit?
    • Potential side effects?
  – Need for non-human Primate Studies
  – Future Clinical Trials
    • Patient selection
    • Large clinical trial?
      – Caution.: Need more careful small exploratory studies