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Cognitive Roles of Anterior and Posterior Pedunculopontine Tegmentum Subregions

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THE ROLE OF ANTERIOR AND POSTERIOR SUBREGIONS OF THE PEDUNCULOPONTINE TEGMENTUM IN SENSORIMOTOR GATING AND ASSOCIATIVE LEARNING

Monograph Article

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Abstract

The pedunculopontine tegmentum (PPT) is part of the mesopontine cholinergic system with distinct anterior and posterior subdivisions. With fast sensory input and descending connections to brainstem locomotor centers, we predict posterior PPT (pPPT) mediates prepulse inhibition of acoustic startle reflex, a form of sensorimotor gating that affects attentional processes. Similar to pPPT cholinergic projections to ventral tegmental area, we predict anterior PPT cholinergic input to substantia nigra regulates dopamine release in striatum, which is important for reinforcement learning. We lesioned the PPT bilaterally in male Sprague Dawley rats with ibotenic acid. Posterior cholinergic cell loss was significantly correlated with prepulse inhibition scores, consistent with our predictions for pPPT mediation of PPI. Anterior cholinergic cell loss was not correlated with performance in cued version of Morris water maze task, though lesions were likely insufficient. These results contribute to investigation of anterior vs. posterior PPT contribution to higher cognitive function.

Keywords

Pedunculopontine tegmentum, acetylcholine, sensorimotor gating, prepulse inhibition, acoustic startle reflex, substantia nigra pars compacta, Morris water maze

List of Abbreviations

ACh	acetylcholine
aPPT	anterior PPT
ASR	acoustic startle response
ChAT	choline acetyl transferase
DA	dopamine
DBS	deep brain stimulation
FOG	freezing of gait
GAD	glutamic acid decarboxylase
$GP_{(i/e)}$	globus pallidus (internal/external)
IBO	ibotenic acid
IC	inferior colliculus
ISI	interstimulus interval
LDT	lateral dorsal tegmentum
LGN	lateral geniculate nucleus
LTH	long-term habituation
NAc	nucleus accumbens
PD	Parkinson's disease
PnC	caudal portion of pontine reticular formation
PI	postural instability
PPI	prepulse inhibition
PPT	pedunculopontine tegmentum
pPPT	posterior PPT
SC	superior colliculus
SNc	substantia nigra pars compacta
STH	short-term habituation
STN	subthalamic nucleus
vAChT	vesicular ACh transporter
vGluT	vesicular glutamate transporter
VP	ventral pallidum

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Chapter 1

1 The Pedunculopontine Tegmentum (PPT)

The pedunculopontine tegmentum (PPT) in rats, known as pedunculopontine nucleus (PPN) in humans, is a widely connected structure that integrates sensory input with motor output. It is part of the mesopontine cholinergic system along with lateral dorsal tegmentum (LDT) and is located in the upper brainstem. The PPT has recently received attention as a candidate for deep brain stimulation (DBS) as a means of treating motor symptoms in Parkinson's disease (PD). In addition to its role in locomotion, the PPT is involved in sensory reception, providing an integration point between sensory inputs and motor outputs. Understanding its function is also important for sensorimotor gating disorders such as schizophrenia.

1.1 Cell Biology

Despite being a cholinergic nucleus, non-cholinergic (glutamatergic, GABAergic) neurons, intermingled and interconnected with cholinergic cells, comprise a majority of PPT neurons (Mena-Segovia et al., 2009; Wang and Morales, 2009). The PPT is an elongated structure that displays anterior-posterior changes in relative abundance of each cell type (Table 1).

Table 1: Distribution (%) of each neuronal subpopulation in anterior, posterior, and whole PPT. The aPPT is more densely populated with GABAergic and Glu neurons; pPPT more densely populated with Glu and cholinergic neurons. Adapted from Wang and Morales, 2009.

	Anterior	**Posterior**	**Total**
Cholinergic	23 ± 2	31 ± 3	27 ± 2
Glutamatergic	37 ± 2	50 ± 4	43 ± 2
GABAergic	40 ± 4	19 ± 2	31 ± 3

GABAergic and glutamatergic neurons are each almost twice as numerous as cholinergic cells in anterior PPT (aPPT); the posterior PPT (pPPT) is more heavily populated by glutamatergic and, to a lesser degree, cholinergic neurons (Mena-Segovia et al., 2009; Wang and Morales, 2009; Martinez-Gonzalez et al., 2011). Further, the difference in GABAergic cell density coincides with a change in the cytoarchitecture and organization of cholinergic neurons (Wang and Morales, 2009). Where GABAergic neurons are dense near anterior regions, cholinergic neurons are bipolar and organized in a layer-like structure; moving posterior, cholinergic neurons become more rounded and randomly organized (vs. layer-like) and have increased number of processes (Wang and Morales, 2009). These anterior-posterior changes in cell type composition as well as distinct connectivity patterns to be discussed below, suggest functional dissociation between anterior and posterior regions of PPT. Despite this dissociation, a large number of PPT projection neurons send axons along rostro-caudal axis of PPT to synaptically link anterior and posterior regions (Mena-Segovia et al., 2008; Ros et al., 2010), suggesting these subdivisions of PPT remain functionally integrated.

PPT neurons characteristically have long-range axons reaching numerous targets across the brain (Woolf and Butcher, 1986). Most cholinergic PPT neurons project to thalamus, with each having roughly 5-6 collaterals innervating basal ganglia, tectum, lower brainstem, and other areas (Mena-Segovia et al., 2008; Martinez-Gonzalez et al., 2011). In contrast, non-cholinergic neurons are generally more restricted in axon length and have only 2-3 collaterals (Ros et al., 2010).

Previous immunolabeling studies raised the possibility that some cholinergic neurons of the PPT co-express glutamate or GABA markers (Clements and Grant, 1990; Lavoie and Parent, 1994; Wang and Morales, 2009), suggesting co-release of ACh with these neurotransmitters. Wang & Morales 2009 investigated this possibility further by using *in situ* hybridization to detect cellular expression of mRNA transcripts of choline acetyltransferase (ChAT), glutamic acid decarboxylase (GAD), and vesicular glutamate transporters (vGluT1, vGluT2, and vGluT3). ChAT is important for synthesis of

acetylcholine and GAD is important for production of GABA. Contrary to these previous studies, Wang & Morales showed that less than 2% of PPT cholinergic neurons expressed vGluT or GAD mRNAs. Although cholinergic, glutamatergic, and GABAergic neurons in PPT are distinct subpopulations, they are intermingled among each other rather than compartmentalized (Wang & Morales 2009).

1.2 Connectivity

Afferent Connectivity

The PPT receives a wide array of input from functionally diverse areas of the brain, including the cortex, thalamus, cerebellum, and basal ganglia (Saper and Loewy, 1982; Semba and Fibiger, 1992). Anterior and posterior regions of the PPT receive input from distinct brain regions.

GABAergic neurons of entopeduncular nucleus in rodents (analogous to globus pallidus internal segment in humans) and of substantia nigra pars reticulata (SNr) preferentially target aPPT, especially non-cholinergic cells (Noda and Oka, 1984; Nakamura et al., 1989; Saitoh et al., 2003; Martinez-Gonzalez et al., 2011). Thus, aPPT receives input from basal ganglia output nuclei.

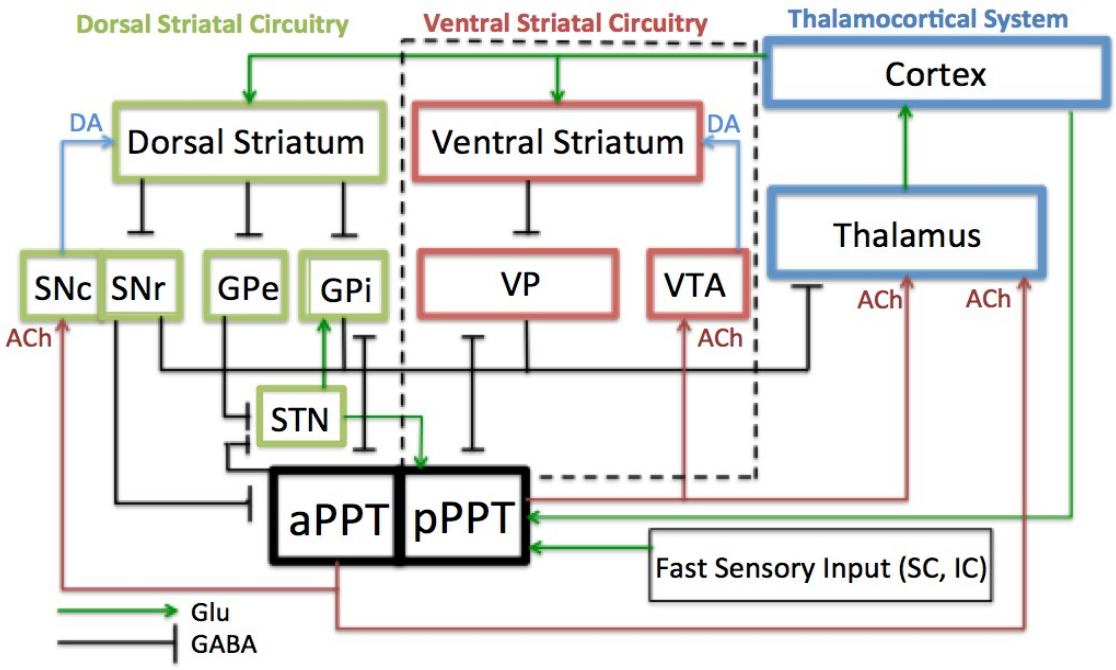

Figure 1: Afferent and efferent PPT interconnectivity with dorsal and ventral striatal circuitry and thalamocortical systems. Cholinergic PPT projections to thalamus make collaterals to DAergic neurons of substantia nigra compacta (SNc) and ventral tegmental area (VTA) from anterior and posterior PPT, respectively. NOTE: Not all connections are shown.

The pPPT receives direct excitatory input from prefrontal and frontal lobe areas involved in motor control (Sesack et al., 1989; Matsumura et al., 2000; Martinez-Gonzalez et al., 2011), excitatory input from subthalamic nucleus (STN, Martinez 2011), and GABAergic input from ventral striatum (Li et al., 2009). In addition, pPPT receives fast sensory input from superior and inferior colliculi (Woolf & Butcher 1986, Martinez 2011) and serotonergic input from dorsal raphe nucleus (Steininger et al., 1997). The DRN typically targets non-cholinergic cells (Steininger 1997). Thus pPPT typically receives both sensory and motor excitatory input, in contrast to largely inhibitory input to aPPT.

In summary, negative inhibitory outflow from globus pallidus (GPi), and excitatory input from motor cortex and STN represent the three major inputs to PPT (Stein and Aziz, 2012). Disrupting balance of these inputs can have pathological implications, as in PD patients. In PD, inhibitory input from GPi is increased significantly (Z. Aziz L.

Davies J. Stein S. France, 1998), resulting in net inhibition.

The PPT also receives DA input, presumably from the mesencephalon (Rolland 2009), and input from deep cerebellar nuclei (Hazrati and Parent, 1992; Martinez-Gonzalez et al., 2011), locus coereleus (Jones and Yang, 1985), lateral dorsal tegmentum (Satoh and Fibiger, 1986), and contralateral PPT (Semba and Fibiger, 1992).

Efferent Connectivity

The PPT provides a substantial amount of cholinergic innervation to the thalamus (Saper and Loewy, 1982; Sugimoto and Hattori, 1984; Hallanger and Wainer, 1988; Martinez-Gonzalez et al., 2011). These thalamic-projecting cholinergic neurons innervate several thalamic nuclei and send collaterals to basal forebrain, pontine reticular formation, superior and inferior colliculi, midbrain dopamine neurons and basal ganglia (Semba et al., 1990; Losier and Semba, 1993; Mena-Segovia et al., 2008; Martinez-Gonzalez et al., 2011), among other brain areas. The PPT also sends non-cholinergic input to the thalamus (Smith et al., 1988; Steriade et al., 1988; Martinez-Gonzalez et al., 2011).

The aPPT sends projections, including collaterals from thalamic-projecting neurons, largely to structures associated with the dorsal striatal circuitry. The aPPT projects to entopeduncular nucleus in rats and cats, analogous to GPi in humans, (Saper and Loewy, 1982; Jackson and Crossman, 1983; Woolf and Butcher, 1986; Charara and Parent, 1994; Shink et al., 1997; Martinez-Gonzalez et al., 2011). In monkeys, retrograde tracing from GPi gives rise to a large number of neurons in aPPT (of which 40% are cholinergic). PPT also interacts indirectly with basal ganglia outflow nuclei through STN, and a smaller number of neurons that project to GPe (Charara & Parent 1994). The aPPT also innervates dopaminergic neurons of substantia nigra pars compacta (SNc). In rat and monkey, aPPT sends glutamatergic and cholinergic input to SNc (Sugimoto and Hattori, 1984; Clarke et al., 1987; Scarnati et al., 1987; Gould et al., 1989; Bolam et al., 1991). These projections emit collaterals that innervate the medial reticular formation in cat (Nakamura et al., 1989). In rats, cholinergic and non-

cholinergic neurons that arborize within SNc primarily arise from ipsilateral aPPT (Lavoie and Parent, 1994; Oakman et al., 1995; Takakusaki et al., 1996; Oakman et al., 1999). In addition to direct and indirect reciprocal interaction with basal ganglia output nuclei, aPPT innervates SNc DAergic cells, suggesting influence on dorsal striatal function.

Thalamic-projecting cholinergic neurons of pPPT emit collaterals to lower brainstem, STN, dopaminergic neurons of VTA, and superior colliculus (SC) and inferior colliculus (IC, Martinez 2011), among others. Descending projections to lower brainstem and spinal cord arise from collaterals of ascending axons in both cholinergic and non- cholinergic neurons (Meno-Segovia 2008), or from non-cholinergic neurons with single descending axons (Ros et al., 2010). Both cholinergic and non-cholinergic neurons innervate the pontine reticular formation in rats (Semba et al., 1990; Takakusaki et al., 1996). These descending connections are considered to be directly involved in locomotion since stimulation of pPPT neurons leads to prolonged activation in nucleus reticularis pontis oralis (affects motor activity and muscle tone during REM) and changes in flexor/extensor nerves (Garcia-Rill et al., 2001). The pPPT also projects to DAergic neurons of VTA; VTA receives substantial ACh input from pPPT in rat and monkey (Sugimoto and Hattori, 1984; Oakman et al., 1995; Charara et al., 1996; Geisler and Zahm, 2005), as well as glutamatergic and GABAergic input (Charara et al., 1996). Innervation of SC and IC by pPPT neurons is mostly ipsilateral and is both cholinergic and non-cholinergic (Beninato and Spencer, 1986; Hall et al., 1989; Mena-Segovia et al., 2009). Thus, pPPT neurons show greater diversity in projection targets and connectivity overall than aPPT. Interconnectivity with thalamocortical systems and DRN suggests a role in modulating brain states (Martinez 2011), while a role in ventral striatal function is suggested by interconnectivity with ventral pallidum and VTA DAergic neurons. Fast sensory input from SC/IC and direct connections to brainstem locomotor regions suggests pPPT may enable incoming sensory info to modify/affect behavioral output, consistent with a role in sensorimotor gating.

In summary, the PPT is a widely connected structure with distinct anterior/posterior

profiles of connectivity. Anterior PPT is more highly integrated with dorsal striatal circuitry and basal ganglia outflow nuclei and has a greater portion of GABAergic neurons. Conversely, posterior PPT has a more diverse connectivity profile suggesting more a more diverse functional role.

1.3 PPT Function

Much investigation has been made into understanding functional role of the PPT. Given interconnectivity with multiple brain networks as well as heterogeneity of neuronal subpopulations, PPT's precise role is likely to be complex and multifaceted, and it has already been attributed multiple functional roles. PPT is generally attributed roles in wakefulness/arousal, sensorimotor gating, reward learning, and locomotion, among others (Ye et al., 2010; Yeomans, 2012).

As part of reticular activating system, PPT is important in modulating REM sleep and wakefulness/arousal (Moruzzi and Magoun, 1949; Hobson and Pace-Schott, 2002; Ye et al., 2010). This function of PPT is mediated largely via interaction with thalamocortical systems. These cholinergic inputs facilitate thalamic synaptic transmission, resulting in widespread thalamocortical activation during both wakefulness and REM sleep (Steriade and McCarley, 2005). The subset of cholinergic neurons active immediately prior to onset of REM sleep could be initiating cortical arousal during dreaming (Kayama et al., 1992; Losier and Semba, 1993; Steriade and McCarley, 2005).

Descending connections to pontine reticular formation have been linked to sensorimotor gating of motor responses (Koch et al., 1993; Swerdlow & Geyer 1993; Yeomans et al., 2001; Meno-Segovia et al., 2008; Ros et al., 2010). PPT's receipt of fast sensory input and quick downstream connections to locomotor regions are consistent with role in integrating sensory input with motor output on rapid time scales, important for role in sensorimotor gating (Yeomans et al., 2006; Yeomans et al., 2010).

PPT's interconnectivity with ventral striatal circuitry underlies its importance in reward learning and memory. PPT is essential for forming associations between reward and environmental stimuli, or between actions and rewards (Alderson et al., 2002; Alderson et al., 2004; Alderson et al., 2006; Alderson et al., 2008; Wilson et al., 2009; MacLaren et al., 2013). This role specifically is relevant to drug addiction, and PPT's integration into ventral striatal circuitry is relevant to schizophrenia.

PPT's role in initiation and modulation of gait and other stereotyped movements, stemming from its interaction between basal ganglia and brainstem locomotor regions, underlies its relevance to Parkinson's disease (Pahapill and Lozano, 2000). High frequency PPT stimulation induces akinesia, likely due to inactivation of cells at these frequencies resulting in functional inhibition (Jenkinson et al., 2004; Jenkinson et al., 2009). Conversely, low-frequency stimulation increases motor activity (Florio et al. 2001; Jenkinson et al., 2004; Jenkinson et al., 2009; Ferraye et al., 2010; Khan et al., 2012; Stein and Aziz, 2012). Stimulation of PPT can elicit organized motor programs, although stepping is required as single pulses are insufficient (Garcia-Rill et al., 1983; Garcia-Rill and Skinner, 1988; Garcia-Rill et al., 2001). Stepping-up PPT with high frequency trains of stimulation can have differential effects depending whether current is slowly increased or kept constant. If it is slowly increased, muscle tone increases bilaterally then movement is initiated (Garcia-Rill et al., 1983; Garcia-Rill et al., 2001). If current is kept constant, a startle reflex is induced which may be followed by stepping or turning movements (Garcia-Rill et al., 1983; Garcia-Rill et al., 2001). PPT connectivity with dorsal striatal circuitry and brainstem locomotor regions suggests a role in higher cognitive control of locomotion. In addition to its role in locomotion, PPT modulation of SNc DAergic cells could influence dorsal striatal learning functions.

In the following, the PPT's role in modulating thalamocortical systems will be discussed first to provide a detailed example of ACh mechanism of action. Then, PPT's role in sensorimotor gating of locomotor responses in pontine reticular formation will be addressed. Finally, PPT interaction with both ventral and dorsal striatal circuitry will be considered in the context of learning by these systems.

1.4 Role in Thalamocortical Systems

Along with LDT, PPT is part of the cholinergic arm of the reticular activating system (RAS) and is critical for the switch from non-REM to REM sleep or wakefulness (Hobson and Pace-Schott, 2002). PPT stimulation induces cortical desynchronization similar to activity observed during REM sleep and wakefulness (Moruzzi and Magoun, 1949). This effect is mediated largely by cholinergic PPT projections to thalamocortical relay neurons, which are depolarized by increased concentration of ACh in "specific" and "nonspecific" thalamic nuclei (McCormick, 1992; Kobayashi and Nakamura, 2003; Parent and Descarries, 2008). Depolarization increases excitability of these neurons to incoming sensory input, which at behavioral level increases awareness of environmental information. Both specific and nonspecific thalamic nuclei are involved in sensory processing and, presumably, modulation of the conscious experience through thalamocortical 40Hz rhythm (Llinas and Ribary, 2001). Increase in thalamic ACh concentration also inhibits reticular thalamic neurons, blocking spindle oscillations and delta waves that appear during non-REM sleep (McCormick, 1992). Together, these actions promote a state of higher conscious awareness. These actions illustrate role of ACh, PPT and thalamocortical systems in the reception of sensory information and awareness of environmental surroundings.

ACh projections to lateral geniculate nucleus (LGN) in the thalamus provide a good example of mechanism of influence on thalamic nuclei (McCormick and Prince, 1987; McCormick and Pape, 1988; Uhlrich et al., 1995; Sherman, 1996; Kobayashi and Isa, 2002). ACh depolarizes thalamocortical relay neurons via nicotinic ACh receptors (nAChRs) and M1 type muscarinic ACh receptors (mAChRs), and hyperpolarizes GABAergic interneurons via M2 mAChRs (Sherman, 1996). Continuous depolarization by ACh activates low-threshold Ca^{2+} channels, changing firing mode from bursting to more regular pattern; the latter firing mode is necessary for visual processing in cortex (Sherman 1996). Activating PPT neurons enhances visual responses in LGN without changing receptive field size by making these neurons more excitable, suggesting PPT could enhance the contrast of a visual scene during early processing, in effect increasing

signal-to-noise ratio of sensory input (Uhlrich et al., 1995; Kobayashi and Isa, 2002). It should also be noted that the PPT provides non-cholinergic input to the thalamus as well (Smith et al., 1988; Steriade et al., 1988).

While this research does not focus on PPT's role in ascending reticular activating system and thalamocortical circuitry, these thalamic projections are important to consider when investigating other functions since most PPT connections to other regions are collaterals of these neurons. Collateralization of these thalamic-projecting neurons to motor systems underlies the PPT's integrative function, which often confers information on timing, or is related to timing, of sensory input.

1.5 Role in Prepulse Inhibition

The caudal portion of the pontine reticular nucleus (PnC) is a part of the brainstem with different locomotor programs, including those neurons necessary for inducing the acoustic startle response (ASR) (Yeomans and Frankland, 1995).

The Acoustic Startle Response

The ASR is a whole-body flinch induced by a sudden loud acoustic stimulus that sufficiently depolarizes giant neurons of PnC (Koch, 1999). This response is typically believed to be defensive rather than protective (Turpin, 1986; Koch, 1999) since it is difficult to see how widespread flexor contractions offer protection (Graham, 1979).

ASR has a short, well-defined circuit and can be elicited behaviorally in various testing paradigms, providing a good model for studying sensorimotor gating mechanisms (Koch et al., 1999). It is present across species and is subject to different forms of sensorimotor gating, including habituation and prepulse inhibition (Prosser and Hunter, 1936; Pfeiffer, 1962; Russell, 1974; Currie and Carlsen, 1985; Baird et al., 1993;

Wicks et al., 1996; Mongeluzi et al., 1998; Zottoli et al., 1999).

Habituation

Habituation is the decline in ability of repeated stimuli to elicit a response or activate the sensorimotor machinery (Geyer et al., 1990). Habituation is among the most basic forms of non-associative learning (Geyer et al., 1990). From an evolutionary perspective, the ability to filter out repeated stimuli that pose no danger enables central processing machinery to focus on novel and potentially harmful environmental cues, and thus habituation is a form of filtering out and ignoring irrelevant information (Leaton et al., 1985; Pilz and Schnitzler, 1996; Koch, 1999).

Habituation can occur over both short and long time frames. Short-term habituation typically occurs on scale of seconds to minutes within a testing session, whereas long-term habituation typically occurs over days, across testing sessions (Leaton et al., 1985; Pilz and Schnitzler, 1996; Koch and Schnitzler, 1997; Koch, 1999; Quednow et al., 2004). In context of the ASR, short-term habituation occurs within first 30 startling pulses, most significantly in early trials (Leaton et al., 1985; Pilz and Schnitzler, 1996). Stimuli become less potent at eliciting a response the more often they are repeated, although a floor effect is observed at which point startle response does not habituate further (Leaton et al., 1985; Pilz and Schnitzler, 1996).

While short-term habituation is mostly reversed within minutes to hours, there is typically a slight decrease in initial startle amplitude each successive day of exposure which is due to long-term habituation (Leaton et al., 1985; Pilz and Schnitzler, 1996). As animals become more familiar with context in which stimuli are presented (i.e. startle boxes), stimuli are less able to elicit startle response.

Short- and long-term habituation are based on different mechanisms. While habituation is not generally attributed to the PPT, it is important to consider when studying sensorimotor gating of ASR as both forms occur during the testing paradigm. However,

vAChT KO mice have no long-term habituation (Azzopardi et al., 2013), so cholinergic mechanisms might be involved and the role of PPT in long-term habituation therefore cannot be excluded.

Prepulse Inhibition

The delay between the time sensory information stimulates peripheral sensory receptors to the time it reaches attention creates a window for new sensory information to redirect attention and overtake the information processing machinery. Sensorimotor gating mechanisms exist to protect the processing of the original stimulus by suppressing processing of subsequent stimuli, ensuring potentially dangerous information reaches the attention of animal (Graham, 1975). Weakness or failure of these gating mechanisms is a hallmark of some disorders, including schizophrenia, and can lead to sensory overload (Graham, 1975; Braff et al., 1978). Prepulse inhibition (PPI) is a form of this gating that prevents incoming sensory information from activating automatic motor responses, such as an acoustic startle reflex (Graham, 1975; Braff et al., 1978). Typically this form of gating lasts between 30-500ms following sensory input (Hoffman and Ison, 1980), which corresponds to length of pre-attentive state (Graham, 1975; Braff et al., 1978; Ellenbroek, 2004). The ability of sudden loud incoming acoustic stimuli to activate a startle response is reduced when preceded by non-startle-inducing acoustic stimulus within the time frame of PPI. The initial prepulse prevents subsequent startle-inducing stimulus from fully activating the startle machinery. The degree to which the startle response amplitude is inhibited is an operational measure of degree of sensorimotor gating (Braff and Geyer, 1990; Swerdlow and Geyer, 1993). PPI is not learned, as it is present upon first presentation of prepulse preceding a startle stimulus, and it does not demonstrate habituation or extinction, suggesting it is an intrinsic behavioral gating process (Russo et al., 1975).

While PPI is not learned and does not exhibit habituation or extinction, it is modulated by higher structures. PPI deficits are present in diseases such as schizophrenia (Braff et al., 1978; Braff and Geyer, 1990; Braff et al., 1992), Huntington's disease (Swerdlow et al.,

1991), autism (Csomor et al., 2008), and Parkinson's disease (Valls-Sole et al., 2004; Perriol et al., 2005), among others. These deficits reflect dysfunction in circuits that modulate sensorimotor gating and consequently manifest in downstream effects such as impairments in PPI. PPI is significantly impaired by excess dopamine (DA) in ventral striatum, suggesting DA activity influences PPI (Swerdlow et al., 1990; Swerdlow et al., 1992). Schizophrenic patients also show elevated ventral striatum DA levels, potentially explaining their deficits in PPI (Perez-Costas et al., 2010; Simpson et al., 2010). Since PPI is an operational measure of sensorimotor gating as a whole, PPI deficits reflect impairments in central gating mechanisms. Deficits in sensorimotor gating can negatively influence attentional processes and consequently result in impairments of higher cognitive functions.

Involvement of PPT

PnC giant neurons are inhibited by application of carbachol, an agonist for both nAChRs and mAChRs (Bosch and Schmid, 2006; Bosch and Schmid, 2008), and muscarinic receptors are believed to mediate startle inhibition between 100 and 500ms following prepulse stimulus, with nicotinic receptors mediating PPI earlier than this (Yeomans et al., 2010). These results suggest cholinergic input may provide inhibitory input to PnC giant neurons. This cholinergic input is believed to arise from PPT, which is part of parallel inhibitory pathway alongside excitatory startle circuit (see Figure 2). However, there is also non-cholinergic input from PPT to PnC and this may also play a role in PPI. Previous studies have not been able to lesion cholinergic neurons selectively and thus non-cholinergic cell loss must be considered.

Incoming acoustic stimuli activate the cochlear root, which activates giant neurons of the PnC; strong enough activation activates motor neurons to elicit startle response (Fig 2). Parallel to this hypothetical primary pathway is an inhibitory PPI pathway that includes inferior colliculus (IC) and PPT (Koch et al., 1993; Swerdlow and Geyer, 1993; Koch, 1999; Yeomans et al., 2006). Both bilateral IC lesions and bilateral, whole PPT lesions have been shown to separately block PPI (Leitner and Cohen, 1985; Koch et al., 1993; Swerdlow and Geyer, 1993), and PPT stimulation inhibits giant neurons in

the PnC (Yeomans et al., 2001; Bosch and Schmid, 2008). The superior colliculus (SC) is connected to this inhibitory pathway in parallel, supported by failure of SC lesions in blocking PPI of ASR (Fendt et al., 2001; Yeomans et al., 2006).

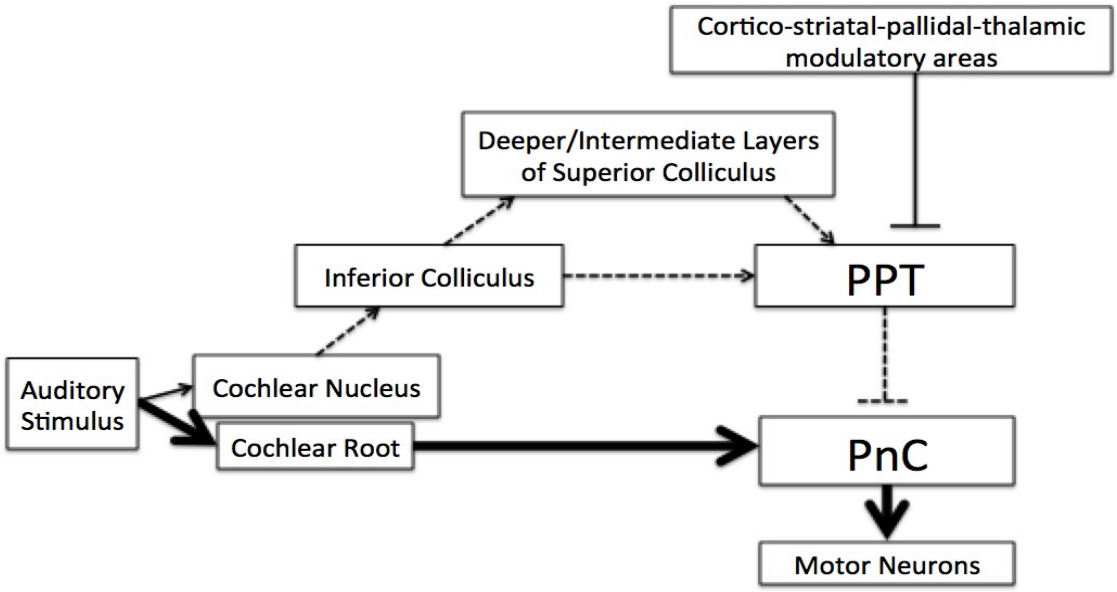

Figure 2: Primary acoustic startle pathway (bold) and proposed parallel inhibitory prepulse inhibition pathway (dotted). Activation of inhibitory pathway by prepulse limits ability of subsequent startling pulse to depolarize PnC giant neurons. Adapted from Yeomans, 2012.

Modulation of PPI by higher brain structures is mediated via connections to PPT (Yeomans et al., 2006; Yeomans, 2012). While strong evidence implicates both PPT and ACh separately in PPI, loss of non-cholinergic PPT neurons in lesion studies and the ability of GABA to inhibit PnC giant neurons raise the possibility that PPI mechanisms may be more complex than direct ACh inhibition of startle mediating PnC neurons by the PPT (Koch et al., 1993; Swerdlow and Geyer, 1993; Bosch and Schmid, 2006; Bosch and Schmid, 2008; Yeomans et al., 2010).

The purpose of this study was to determine whether the PPT's role in prepulse inhibition is localized to posterior regions. Previous studies used whole PPT lesions (Koch et al., 1993; Swerdlow & Geyer 1993). Given its projections to downstream brainstem

locomotor regions as well as receipt of fast sensory input, our prediction is that the PPT's role in PPI arises from pPPT neurons. If so, lesions limited to pPPT, rather than whole PPT, should be sufficient to block PPI of the ASR in rats, whereas aPPT lesions should not block PPI.

1.6 Role in Striatal Reinforcement Learning

The PPT is highly interconnected with basal ganglia nuclei. Anterior PPT neurons are more connected with the dorsal striatal circuitry, including the globus pallidum (GP), subthalamic nucleus (STN), and substantia nigra (SN) while posterior neurons are more connected with the ventral striatal circuitry, including ventral pallidum (VP) and ventral tegmental area (VTA) (Sugimoto and Hattori, 1984; Lavoie and Parent, 1994; Oakman et al., 1995; Charara et al., 1996; Takakusaki et al., 1996; Oakman et al., 1999; Geisler and Zahm, 2005). Of particular interest to this research is the PPT cholinergic modulation of SNc/VTA DAergic activity and the role these connections play in striatal reinforcement learning.

PPT Cholinergic Regulation of Midbrain Dopamine Neurons

The PPT sends both cholinergic and glutamatergic fast excitatory input to midbrain dopamine neurons to generate burst firing (Clarke et al., 1987; Futami et al., 1995; Forster and Blaha, 2003). These DA neurons are located in the ventral tegmental area (VTA), which is connected with ventral striatal circuitry, and in the substantia nigra pars compacta (SNc), which is part of dorsal striatal circuitry. VTA is innervated by pPPT whereas aPPT innervates SNc (Sugimoto and Hattori, 1984; Lavoie and Parent, 1994; Oakman et al., 1995).

Stimulation of the PPT results in monosynaptic excitation of DA neurons in SNc or VTA (Lacey et al., 1990). These ACh inputs are essential for inducing and maintaining burst firing in DA neurons (Floresco et al., 2003; Lodge and Grace, 2006).

Stimulation of PPT induces tri-phasic DA release pattern in the striatum. Initially there is a fast increase in DA efflux in the NAc/striatum for 1-3 minutes due to nicotinic and glutamate receptors in VTA or SNc (Forster and Blaha, 2000). As M2-like inhibitory muscarinic autoreceptors in PPT reduce cholinergic activation of DA neurons, there is a net decrease from baseline of DA efflux in NAc/striatum for 2-8 minutes following stimulation (Forster and Blaha, 2000). Following this, there is a second wave of sustained DA efflux 8-60 minutes after PPT stimulation mediated by M5 receptors, which are slower to bind ACh; this prolonged wave of DA efflux is abolished by blocking M5 (Yeomans et al., 2001; Forster et al., 2002). M5 receptors are important for rewarding effects of hypothalamic stimulation, opiates, ethanol, and carbachol in VTA (Yeomans, 2012). Antagonism of muscarinic receptors in VTA reduced rewarding effects of both hypothalamic stimulation and food in rats (Yeomans et al., 1985; Kofman et al., 1990; Sharf et al., 2005; Sharf and Ranaldi, 2006).

PPT neurons are believed to provide an error prediction signal, necessary for reinforcement learning, to midbrain DA neurons (Kobayashi and Okada, 2007). The reinforcement learning process between PPT and DA neurons has been simulated (Brown et al., 1999; Dranias et al., 2008). During early trials with frequent errors and unpredictable rewards, DA neurons are activated by rewards, causing DA burst in striatum (dorsal or ventral); as performance improves (fewer errors) and rewards become more predictable, DA neuron activation by primary rewards is progressively reduced (Brown et al., 1999). Primary reward signal may originate from PPT whereas suppression of DA neuron response to reward could originate from GABAergic neurons of striosomes in dorsal or ventral striatum (Gerfen, 1992; Kobayashi and Isa, 2002). Thus, PPT provides a fast excitatory signal for primary reward, and the striatum learns stimulus-reward/stimulus-response association by progressively inhibiting phasic activation of DA neurons by primary reward, until it completely eliminates any DA burst in striatum. Furthermore, if reward is expected but not received, GABAergic neurons fire but PPT does not, causing net inhibition of SNc and a DA dip in the striatum (Dranias et al., 2008). Both simulations accurately predicted electro-physiological neural activity

during behavioral tasks in monkeys (Brown et al., 1999; Dranias et al., 2008). Thus, PPT relays excitatory component of tonic stimulus/reward prediction – phasic primary reward signals – to DA neurons to induce burst firing (Kobayashi and Okada, 2007).

PPT Role in the Ventral Striatal Circuitry

The pPPT is integrated with the ventral striatal circuitry and has consistently been implicated in reward-reinforcement learning (Bechara and van der Kooy, 1989; Olmstead and Franklin, 1993; Laviolette and Van, 2004). Of particular importance to this form of learning are PPT cholinergic projections to DAergic neurons of the VTA, which provide both tonic and phasic modulation (Clarke et al., 1987; Futami et al., 1995; Forster and Blaha, 2003). Recent studies have focused on using excitotoxins to create bilateral lesions of either aPPT or pPPT. One recent study investigated effects of these lesions on ability of animals to learn bar pressing task on variety of fixed-ratio and variable-ratio reinforcement schedules. Rats with pPPT lesions were significantly slower than sham or aPPT-lesioned animals at learning the association between bar-pressing and food reward (Wilson et al., 2009). In another study, rats either received or did not receive operant training with food prior to whole-PPT lesion surgery (Alderson et al., 2004). Pre-trained animals performed no different than controls in a low fixed-ratio (FR2) intravenous self- administration of d-amphetamine paradigm after lesion surgery, whereas naïve rats made significantly fewer infusions (Alderson et al 2004). Both pre-trained and naïve rats made significantly fewer infusions on a progressive ratio (PR5) schedule relative to controls, suggesting that PPT is important for both forming and updating associations (Alderson et al 2004). These FR2 results also indicate that the PPT is largely unnecessary for retrieval of unchanged associations, suggesting that the role of PPT is largely focused on acquisition rather than maintenance/retrieval of associative memory. If PPT-lesioned animals (no pre-training) were provided non-contingent dose of d-amphetamine at the start of each session, they performed similar to controls in both FR2 and PR5 paradigms, suggesting observed deficits may result from striatal DA transmission (Alderson et al., 2004). In a follow-up study, these reward-learning functions were localized to posterior regions of PPT and their influence of VTA

DAergic cell firing (Alderson et al., 2006). Thus, pPPT regulation of VTA DA neuron firing is essential for formation of action- reward associations and plays an important role in reinforcement learning in the ventral striatal network.

PPT Role in the Dorsal Striatal Circuitry

The PPT's interaction with the dorsal striatal circuitry underlies its relevance to the pathophysiology of Parkinson's disease (PD). Some of the most debilitating symptoms of PD include gait initiation failure, freezing of gait (FOG), and postural instability (PI), yet these locomotor akinesia symptoms often do not respond to traditional DA treatment or deep brain stimulation (DBS) of basal ganglia nuclei (van Nuenen et al., 2008; Stein and Aziz, 2012). In PD brains, terminals from GPi to PPT neurons show the highest uptake of deoxyglucose, suggesting overactive synapses and a pathological inhibition of PPT (Mitchell et al., 1989; Stein and Aziz, 2012), thus preventing activation of brainstem locomotor programs and causing locomotor akinesia. Furthermore, since overactive GPe (less inhibition of indirect pathway) increases the inhibition of STN, PPT would also lose this excitatory drive (Florio et al., 2001; Jenkinson et al., 2009). Injection of GABA antagonist into PPT reverses akinesia (Nandi et al., 2002). Furthermore, PPT lesions cause locomotor akinesia in monkeys (Munro-Davies et al., 2001), which may be partially relieved by stimulation of remaining PPT neurons at low frequencies (Jenkinson et al., 2004). These data support the PPT as a candidate target for deep brain stimulation to treat some cases of PD. Specifically, PPT DBS is beneficial for patients with FOG/PI symptoms, whereas GPi/STN stimulation is more beneficial for patients with tremors and dyskinesias (Stein and Aziz, 2012). Co-stimulation of STN and PPT increases efficacy of treatment, although the high frequency stimulation required for STN stimulation (130Hz) worsens gait if applied to PPT (Jenkinson et al., 2009), while lower frequency PPT stimulation is beneficial (Ferraye et al., 2010; Khan et al., 2012). The efficacy of PPT stimulation in relieving locomotor akinesia underscores PPT's role in locomotion and in providing higher brain areas access to locomotor machinery. While some groups found slight motor impairment in aPPT-lesioned rats (Alderson et al., 2008), others failed to replicate

this finding (Wilson et al., 2009; MacLaren et al., 2013). Since whole-PPT lesions also fail to impair motor function (Inglis et al., 1994; Winn, 2006, 2008), aPPT lesions should not cause locomotor deficits.

In addition to motor symptoms, it is possible that pathological PPT inhibition or PPT degeneration is also responsible for some cognitive impairments of PD (Rinne et al., 2008). A large subset of PD patients suffers from cognitive decline in addition to motor symptoms (Gotham et al., 1988; Poewe et al., 1991; Feigin et al., 2003; Decamp and Schneider, 2009; Jahanshahi et al., 2010; Takakusaki et al., 2011). PPT's role in sleep-wake cycle may be perturbed in PD patients, who often suffer sleep disturbances years before motor symptoms develop (Ferini-Strambi and Zucconi, 2000; Askenasy, 2001; Larsen and Tandberg, 2001; Jubault et al., 2009; Takakusaki et al., 2011). PD patients also show symptoms of depression, which may be responsive to cholinergic but not serotonergic treatment, suggesting a cholinergic role in PD depression (Wientraub et al., 2005). Many PD patients also show sensorimotor gating deficits with impairments in PPI (Valls-Sole et al., 2004; Perriol et al., 2005). Many of these symptoms could be related to pathophysiology of PPT neurons, explaining lack of responsiveness to DAergic treatment. While PD patients often present clinically with motor impairments, they retroactively report years of non-motor symptoms (Bohnen and Albin, 2011). Many PD patients also suffer from dementia as they lose higher cognitive functions (Lieberman, 2006). Degeneration/inhibition of PPT neurons may be important in development of these higher cognitive deficits (Bohnen et al., 2011). Low-frequency DBS of PPT significantly improved executive functions and working memory and beneficially alleviated sleep architecture (Alessandro et al., 2010). The purpose of this research is to investigate PPT role in higher cognitive functions of dorsal striatum.

While much progress has been made in understanding how pPPT cells influence reward-reinforcement behavior, less is known about aPPT-SNc connection influence on higher cognitive functions. In contrast to impaired performance of pPPT-lesioned rats in fixed (FR2) and progressive (PR5) bar-pressing task, aPPT-lesioned rodents did not display

evident learning deficits in these paradigms, but displayed both anticipation- and perseveration-like behaviors (Wilson et al., 2009). During trials prior to reward delivery signal, aPPT-lesioned animals checked for food significantly more often than other groups, suggesting high anticipation/motivation for reward. Following signal that indicated reward delivery, aPPT-lesioned rats made significantly more bar presses before collecting food than either sham or pPPT-lesioned animals, suggesting perseverance. Together, these contrasting behaviors reflect a disorganized response control (Wilson et al., 2009).

Since pPPT-VTA connections have been implicated in ventral striatal reinforcement learning, the aPPT-SNc connection is a natural candidate for providing error prediction signal necessary for dorsal striatal reinforcement learning. Egocentric (self-oriented) navigation requires an intact dorsal striatum and striatal DA transmission (Lee et al., 2008; Braun et al., 2012). Dorsal striatum lesions impair performance in cue-response and cue-driven navigation tasks (Packard and McGaugh, 1992, 1996; Lee et al., 2008), which may be probed with a cued version of Morris water maze. Unlike the classic spatial version, the cued task requires the animal to rely on intra-maze cue that varies in spatial position every trial and is never located in same place twice. This requires egocentric learning rather than allocentric learning, and is dependent on integrity of dorsal striatum (Packard and McGaugh 1992, 1996; Lee et al. 2008). Rodents with lesions in the dorsal striatum show impairments learning association between the cue and platform, but do not show deficits in the classic spatial version of Morris Water maze that relies on hippocampal allocentric strategies and extra-maze spatial cues (McDonald and White, 1994; Gengler et al., 2005; Lee et al., 2008). Learning to associate a specific cue with the escape, rather than spatial location, requires action-outcome reinforcement learning, which would be dorsal striatum- and DA-dependent (Packard and McGaugh, 1992; Compton, 2004; Kobayashi and Okada, 2007). Similar to how pPPT neurons induce burst firing in VTA neurons to signal primary reward, aPPT neurons could signal desired outcome (reaching platform) by inducing burst firing in SNc neurons, causing DA efflux in striatum. GABAergic input from the dorsal striatum would progressively counteract excitatory drive by

aPPT neurons as animal learned to predict it would reach the platform as it approached the cue. Animals with lesions of aPPT would lose this excitatory drive necessary for dorsal striatal reinforcement learning, and thus should display deficits in cued version of the Morris water maze but not in the classic spatial version. Animals with pPPT lesions should not show deficits in either task.

1.7 Objectives, Hypotheses, and Approach

Objectives

1. To confirm that PPT is not important for spontaneous locomotion in rats
2. To evaluate contribution of anterior and posterior PPT regions to prepulse inhibition of the acoustic startle response
3. To evaluate contribution of anterior and posterior PPT regions to dorsal striatal reinforcement learning

Hypotheses

1. We hypothesize that although PPT stimulation can elicit coordinated locomotor programs in rats, it is not important for spontaneous locomotion
2. We hypothesize that neurons of posterior PPT mediate prepulse inhibition of the acoustic startle response via projections to the caudal portion of the pontine reticular formation (PnC)
3. We hypothesize that projections from anterior PPT to dopaminergic neurons of substantia nigra pars compacta are important for dorsal-striatal reinforcement learning by properly timing phasic release of dopamine in striatum

Approach

To explore these hypotheses, we will perform lesion surgery on male rats to bilaterally destroy anterior or posterior PPT cells. After recovery, we will test spontaneous locomotor activity, prepulse inhibition of the acoustic startle response, and dorsal striatal reinforcement learning in a cued Morris water maze task. Histological quantification of lesions will allow behavioral results to be correlated to extent of lesion damage.

Chapter 2

2 Methods

2.1 Animals: Housing and Handling

All animals were male Sprague Dawley rats ordered from Charles River® (Montreal, Quebec) at approximately 300g. Prior to surgery, animals were group housed for one week in clear plastic caging with *ad libitum* access to rat chow and water. Rats weighed approximately 350-400g at the time of first surgery. After first surgery, they were housed individually for duration of protocol to prevent injury. Rats were held in a 23^{o}C temperature-controlled room on a 12:12 hour light-dark cycle with interchanges at 7am and 7pm. Rats were handled for 5 min on day after their first surgery, day after second surgery, and for three consecutive days prior to behavioral testing to habituate rats to handling, familiarize the handler, and minimize anxiety following two surgeries. Rats were tickled to increase affinity for handler (Burgdorf and Panksepp, 2001), and were considered fully handled when they allowed prolonged tickling of belly. Rats were handled throughout testing to maintain familiarity with handler.

2.2 Stereotaxic Surgery

In each rat, two surgeries to lesion right and left PPT separately were performed 8 days apart to maximize survival. One week after arrival, animals underwent unilateral injections targeting aPPTg or pPPTg. Ibotenic acid (IBO), an NMDA-analogue excitotoxin, was used as lesioning agent. IBO is a commonly used excitotoxin in PPT-lesion studies (Jarrard, 1989; Deurveilher and Hennevin, 2001; Florio et al., 2001; Alderson et al., 2002; Alderson et al., 2004; Alderson et al., 2006; Wilson et al., 2009; MacLaren et al., 2013); like other excitotoxins, it has the advantage of sparing fibers passing through the PPT but it is not selective for any particular cell type (Dunbar et al., 1992). We used IBO to be consistent with other groups currently investigating PPT.

Rats were weighed prior to surgery, and were anesthetized via inhalation of 2% vaporized isoflorane (Forane) and 98% oxygen delivered to a nose cone on stereotaxic apparatus (Stoelting). Once anesthetized but before surgery, rats received intra-peritoneal injections of 0.05 mg/kg buprenorphine (Talgesic) and 2.5 mg/kg ketoprofen (Anafen) for analgesia. Rats were fixed in a stereotaxic apparatus (Stoelting) with blunt-ended earbars and their eyes were covered with LacriLube®. The skull was then shaved and cleansed with soap, 70% ethanol, and finally iodine paint (Betadine) before a 3cm mid-sagittal incision was done to allow retraction of skin and exposure of skull's anatomical points: bregma and lambda. The nosepiece was adjusted to keep bregma and lambda at same height, ensuring flat skull. Coordinates were measured from bregma: aPPTg (DV: -0.77 mm, from skull surface, ML: +0.19 mm, from midline; RC: -0.72 mm, from bregma) and pPPTg (DV: -0.72 mm, from skull surface, ML: +0.17 mm, from midline; RC: -0.82 mm, from bregma). The tip of injection needle (Hamilton) was slowly lowered through holes made by engraving drill (RWD Life Science). Infusions of ibotenate (Sigma; made up as 0.063 M solution in phosphate buffer [pH 7.4]) were delivered at rate of 100nL/min for a total injection of 400nL. Control rats received same volume of phosphate buffer only, delivered at same rate. The needle was left at site for 5 min following injection to ensure proper diffusion. Silk sutures were used to close wound, and subjects were given injections of 5mL/kg of 0.9% sterile saline to ensure proper hydration during recovery. Animals were allowed to heal for 8 days before their second surgery, which was identical to the first but on contralateral side. Subjects were also weighed before second surgery. Sutures were cut and removed, and incision line from first surgery was used as incision line for the second. All procedures were approved by the University of Western Ontario Animal Use Committee, and complied with ethical guidelines of Canadian Council on Animal Care involving vertebrate animals in research.

2.3 Behavioral Paradigms

Rats were allowed to recover for two weeks following their second surgery prior to any testing. All behavioral tests were run between 1pm to 5pm.

Locomotor Boxes

Locomotor boxes (Med Associates) were used to assess basic locomotion and habituation of emitted behaviors. Boxes were 45cm x 45 cm x 30 cm and contained photo-beams around all sides to track animal locomotion. Animals were placed in boxes and left for 30 min for 5 consecutive days. Ambulatory distance, average velocity, vertical count (rearings) and stereotypic count (grooming behaviors) were recorded.

Acoustic Startle Boxes

Acoustic startle boxes (Med Associates) were used to assess short- and long-term habituation of acoustic startle response and prepulse inhibition of this elicited behavior. Each startle box consisted of sound-insulated chamber with loudspeaker and platform that constituted a piezoelectric transducer. Cylindrical Plexiglas rat chambers were fixed to the top of the platform, with holes in the side facing the loudspeaker to maximize delivery of acoustic stimuli. Movement of the platform was transduced to electrical signal and converted to arbitrary magnitude, which was proportional to startle response of animal. A "gain" dial allowed this signal to be attenuated (gain<1) for rats with strong startle responses or enhanced (gain>1) for rats with weak startle responses, ensuring signal remained within the range of detection. For three consecutive days prior to testing, rats were acclimated to startle boxes by placement in Plexiglas rat chambers for 5 min of 65dB white noise. On third acclimation day, this was followed by an Input/Output (I/O) function paradigm to determine appropriate gain for remainder of startle testing. In I/O test, 12 stimuli were delivered 30s apart; first stimulus was 65dB (same as white noise) and each subsequent stimulus increased by 5dB. Background noise of 65dB played for the duration of test. Testing paradigm began on day following I/O test. Rats were tested for five consecutive days in same paradigm, which consisted of three consecutive and continuous phases: acclimation, block 1, and

block 2. During acclimation phase, animals were exposed to chambers and 65 dB background noise for 5 min. Background noise played for duration of experiment. 'Pulse-alone' trials delivered only startle-inducing (105dB, 20ms duration) stimuli, while 'prepulse-pulse' trials delivered sub-threshold (i.e. not startle-inducing) prepulses at specific short intervals, or interstimulus interval (ISI), before startle pulse. Prepulse levels used were 75dB and 85dB, and ISI levels used were 10, 30, and 100ms. Block 1 consisted of 33 pulse-alone trials to fully habituate animals before testing for prepulse inhibition. Block 2 consisted of 70 trials pseudo-randomized with 10 pulse-alone trials and 10 trials for each of 6 possible prepulse-ISI combinations. Inter-trial intervals were randomized between 15-30s.

Morris Water Maze

A cued version of Morris Water Maze (Hastings, 150cm diameter) was used to assess egocentric spatial navigation and memory (Packard and McGaugh, 1992; 1996; Lee et al., 2008; Braun et al., 2012). An escape platform was submerged 2cm below the water surface. A yellow cue ball, fixed to a stand, was attached to escape platform surface and extended above water surface to serve as cue signaling platform location. Rats were tested over five consecutive days with four trials each day. The platform was located at different position every trial and its location was never repeated. Rats were guided to platform if they did not find it within 90s. Following five days of cued maze testing, rats were then subject to two days of classical spatial Morris Water maze to probe allocentric spatial navigation/memory. Platform location was randomized among rats but remained constant for a given rat, with locations matched between control and lesion animals. There was no platform cue for spatial paradigm. There were four trials each day. Inter- trial intervals for both paradigms were ~45 minutes apart. Latency and distance to target were assessed as measurements of maze performance.

2.4 Data Analysis

Lesion Classification and Statistics

NADPH stains were used to quantify extent of lesion for each animal (see "Histology", next section, for NADPH staining procedure). Cell counts of cholinergic cells were done in brain slices corresponding to figures in Paxinos & Watson (2006) rat brain atlas, which was used to distinguish aPPT (Figs 92-96) and pPPT (Figs 97-100). The number of cholinergic cells in aPPT and pPPT in control brain slices was averaged and used as the standard number of cholinergic PPT cells. Cholinergic cells in brain slices from each IBO-injected rat were also counted, and individually compared to the standard number of cells for either aPPT or pPPT. Lesion scores were calculated bilaterally:

$$\frac{\#\ cells\ in\ IBO\ brain}{standard\ \#\ of\ PPT\ cells}$$

We also calculated lesion scores for control animals, since it is possible for animals to have higher than the standard number of cells (and thus lesion scores > 1). A lesion score of 1 represents no cell loss (no lesion) while lesion score of 0 represents complete cell loss (full lesion). We took the lower (i.e. greater lesion) of the two lesion scores for each animal and to control for unilateral lesions, we required a threshold of at ~30% bilateral cell loss for inclusion in lesioned group analysis. There were five animals in posterior group that satisfied this criterion. Unilateral lesions (one side <20% cell loss) were not included in analysis. Unfortunately, there were no animals in the anterior IBO group that exceeded 30% bilateral lesion. For this reason, we lowered the criterion for anterior groups to at least 20% bilateral lesion, which included four animals. Since these "n" were very low, and since cell loss was variable, we correlated lesion scores with behavioral data to identify relationships that may not be evident with grouped data. For the correlation analysis, we eliminated animals with <20% cell loss on one side if they had ~30+% cell loss on the other side. Since we took the lower of the two lesion scores for each animal, including animals with <20% cell loss on one side and >30% cell loss on the other would overestimate extent of lesion. Animals with <20% cell loss on both sides were still included for correlations, since their higher lesion scores

(smaller lesions) would be similar to saline animals and account for lack of lesion. Animals with <20% cell loss on one side and <30% cell loss on the other were not excluded for correlations since the difference in cell loss between the sides was not large and their lesion score would still indicate relatively small lesions, similar to saline animals.

Grouped data was analyzed either with unpaired t-tests or with repeated measures ANOVA. We then assessed significance of saline lesion scores with behavioral data and IBO lesion scores with behavioral data. Since there was significant overlap between these groups, we assessed the overall correlation between lesion score and behavioral data (i.e. not separating saline and IBO animals). For data that did not satisfy normality requirements, we used Spearman's coefficient for non-parametric tests of significance.

Locomotor Boxes

Locomotion was assessed using only day 1 data, which was divided into 6 consecutive blocks of 5 min each. Average velocity and distance traveled during block 1 on day 1 were each separately correlated to lesion scores.

Acoustic Startle Boxes

The maximum of the first 3 pulse-alone trials of block 1 of day 1 was taken as an animal's initial/baseline startle amplitude. These values were divided by the gain factor to correct for differences in gains between rats. Short-term habituation was measured as the average of the final five block 1 trials divided by the maximum of the first three trials of block 1. Long-term habituation was measured by dividing the maximum of the first 3 pulse-alone block 1 trials of a given day by the maximum for day 1. PPI was calculated using only block 2 data: the average for each prepulse-ISI combination was divided by the pulse-alone value. Subtracting these values from 1 provided a %PPI value for each rat for each prepulse-ISI combination. Baseline startle amplitude, STH score, LTH score, and PPI values were correlated to lesion scores.

Morris Water Maze

ANY-maze software was used for tracking subject performance in both cued and spatial paradigms. Anterior and posterior groups were analyzed separately. Distance and latency to target were measured for all trials. Since most learning occurred on day 1, especially in third and fourth trial, the distance and latency to target in these trials for each animal was correlated to lesion scores and assessed similarly to other data.

2.5 Histology

After completion of testing, subjects were perfused with 4% paraformaldehyde and brains were extracted for histological analysis of lesions. Brains were stored in 25% sucrose until sliced, after which they were stored in cryoprotectant until staining. Brains were cleaned of cryoprotectant in phosphate-buffered saline (PBS) over ten 10-minute washes on lab shaker at 100rpm. Following this, slices were placed in NADPH-diaphorese staining solution (7.5mg reduced NADPH, 1.6mg Nitroblue Tetrazoleum, 80 µL Triton X-100, 150 µL of 1M malic acid, and 1 mL Tris-HCL pH=8.0). Once in solution, plates were placed in bacterial incubator at 37^{o}C for 60 min at 100rpm. Following this, slices were plated and after drying, were placed in ddH_2O for 5 min, then dehydrated in ascending alcohol (70% → 95% → 2x 100% ethanol), differentiated in 50:50 chloroform:100% ethanol, then cleaned in xylene. After cover-slipping, pictures of each brain were taken under a light microscope for comparison between control and lesion animals. Using Paxinos & Watson rat brain atlas (PPT in figures 92 – 100), cell count was done manually for each figure available. Average cell count for each figure in control animals served as standard number of cells for non-lesion brain.

NADPH staining was used for quantification of lesions because it is a robust technique that is selective for mesopontine cholinergic cells (Dun et al., 1994, Leonard et al., 1995). Nitric oxide synthase 1 (NOS1) mimics the actions of NADPH-diaphorese, catalyzing the reaction of tetrazoleum into mono- and di-formazans (Beesley 1995).

Differentiation removes the soluble monoformazans, leaving only the insoluble diformazans that are present only in cholinergic cells in the mesopontine region, so only cholinergic cells are stained (Dun et al., 1994, Leonard et al., 1995, Hars 1999). This technique allows easy delineation of PPT boundaries since only cholinergic PPT cells are stained (Dun et al., 1994, Leonard et al., 1995, Hars 1999). Furthermore, cholinergic cell loss is a conservative proxy for overall cell loss since cholinergic cells tend to be more resistant to IBO than non-cholinergic neurons (Dunbar et al., 1992).

Nissl staining was done to also visualize non-cholinergic cells. This protocol was done immediately following slicing so no storage in cryoprotectant was necessary. After cleaning in xylene, brains were transferred to 100% propanol, then 100% ethanol. Moving through a descending alcohol series to 50% ethanol rehydrated the slices. These were then washed in ddH_2O and stained in thionine for 15 seconds, rinsed in ddH_2O, then dehydrated in an ascending alcohol series to 96% alcohol. Slices were then differentiated in 96% ethanol:acetic acid solution and transferred to 100% ethanol. After ethanol, slices are transferred to 50:50 xylene:terpentine for 15 minutes, then washed in xylene, and then finally cover-slipped.

Chapter 3

3 Results

3.1 Lesion Analysis: Post-Surgery Spontaneous Turning Behavior and Histological Quantification

All animals receiving IBO injections displayed prominent contraversive turning post-surgery (away from lesion side), except right side surgery for AI16 - AI18 (Table 2), immediately upon waking up.

Table 2: Presence of contraversive turning behavior post-surgery in subjects with anterior or posterior, left and right PPT IBO injections. All but 3 animals displayed this behavior following both surgeries.

Anterior IBO Injections	Left	Right	Posterior IBO Injections	Left	Right
AI13	✔	✔	**PI13**	✔	✔
AI14	✔	✔	**PI14**	✔	✔
AI15	✔	✔	**PI15**	✔	✔
AI16	✔		**PI16**	✔	✔
AI17	✔		**PI17**	✔	✔
AI18	✔		**PI18**	✔	✔
AI19	✔	✔	**PI19**	✔	✔
AI20	✔	✔	**PI20**	✔	✔
AI21	✔	✔	**PI21**	✔	✔
AI22	✔	✔	**PI22**	✔	✔
AI23	✔	✔			
AI24	✔	✔			
AI25	✔	✔			

This behavioral measure suggests nearly all animals receiving IBO injections suffered some degree of bilateral PPT cell loss. No animals were eliminated due to this post-surgery behavioral measure because we cannot eliminate the possibility that this behavior was due to swelling/inflammation in area of toxin injection rather than cell death.

In addition to behavioral measure of PPT lesion, quantification was done with NADPH staining (see "Materials and Methods"). This provided a lesion score with which behavioral data could be correlated. Samples of NADPH and Nissl staining are provided in Figure 3. Lesion scores for each animal are presented in Table 3 (Anterior) and Table 4 (Posterior).

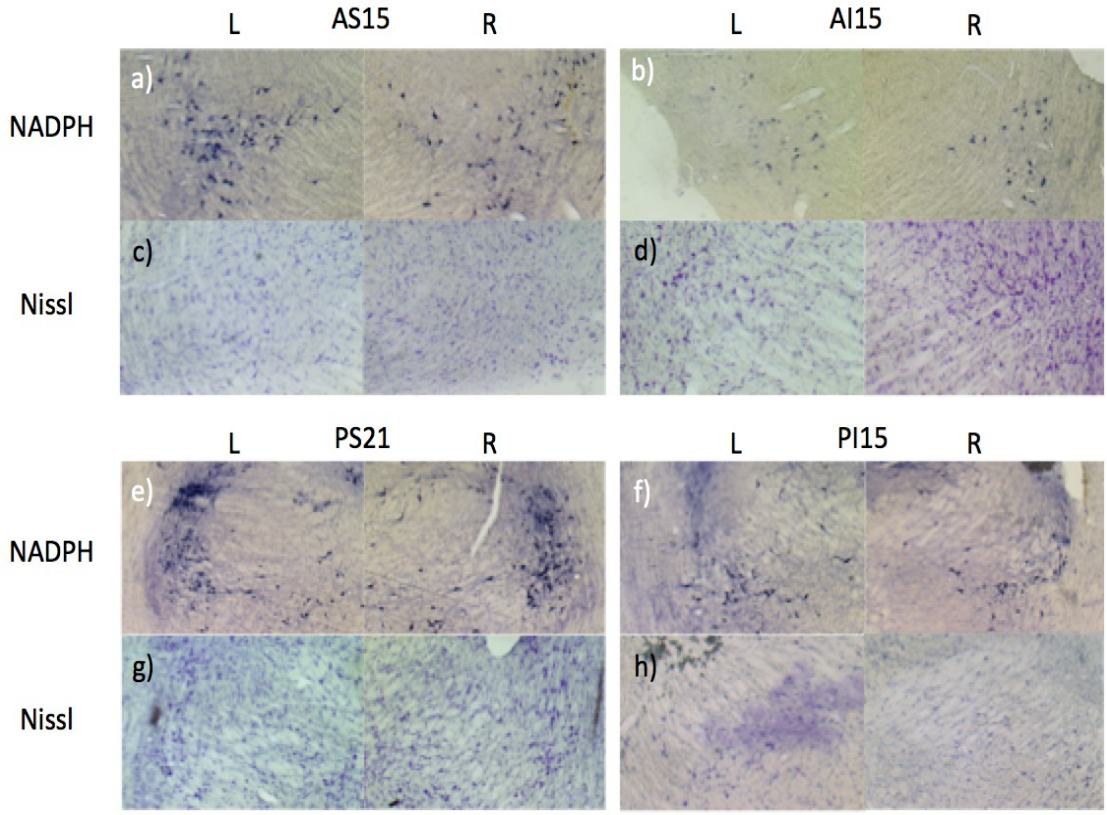

Figure 3: Histology sample from each surgery group (A= anterior, P = posterior, S = saline, I = IBO). Cholinergic cell loss can be observed in IBO subjects with NADPH staining. Non-cholinergic cell loss can be seen with Nissl staining, which stains all PPT neurons.

These figures each represent only one 40µm slice of brain; while AI15 appears to be a bilateral lesion from the above figure, total cell counts across multiple slices of PPT were done to ensure greater accuracy in lesion quantification. Lesion scores account for all slices within aPPT or pPPT.

Table 3: Lesion scores for subjects in anterior injection groups (saline and IBO). Most anterior IBO subjects were not considered to be lesioned. Eight were excluded from grouped analysis due to insufficient cell loss on both sides, and one was excluded for insufficient cell loss on one side. Only this unilateral hit was excluded from correlation analysis.

Anterior Saline	Lesion Score	Anterior IBO	Lesion Score
AS13	0.868	**AI13**	0.974*
AS14	0.906	**AI14**	0.795
AS15	1.168	**AI15**	0.793*
AS16	1.072	**AI16**	0.787*
AS17	0.912	**AI17**	0.810*
AS18	0.940	**AI18**	0.810*
AS19	0.868	**AI19**	0.729*
AS20	0.782	**AI20**	0.896*
AS21	0.895	**AI21**	0.763
AS22	0.985	**AI22**	0.768
		AI23	0.891*
		AI24	0.684*,**
		AI25	0.729

*Excluded from grouped analysis **Excluded from correlation analysis

Table 4: Lesion scores for subjects in posterior injection groups (saline and IBO). Two animals were excluded from grouped analysis because cell loss was sufficient only on one side, and 3 more animals were excluded because cell loss was insufficient on both sides. Only the two with unilateral hits were excluded from correlation analysis.

Posterior Saline	Lesion Score	Posterior IBO	Lesion Score
PS13	0.789	**PI13**	0.746
PS14	0.877	**PI14**	0.682*,**
PS15	0.923	**PI15**	0.390
PS16	0.983	**PI16**	1.101*
PS17	0.853	**PI17**	0.827*
PS18	0.962	**PI18**	0.468
PS19	1.068	**PI19**	0.816*
PS20	1.094	**PI20**	0.439*,**
PS21	0.930	**PI21**	0.625
PS22	0.845	**PI22**	0.641

*Excluded from grouped analysis **Excluded from correlation analysis

Exclusion criteria (described below) were based off lesion scores on both sides of animal. Only animals with sufficient bilateral cell loss were included in grouped analysis. Animals with insufficient cell loss on both sides were included for correlation analysis. Since we used the lower of two lesion scores as an animal's score, we excluded unilateral lesions from correlation analysis as well. See Appendix for table with both lesion scores.

Although there were no lesions (classified as ~30+% cell loss) in anterior ibotenic group, some cell loss was observed (Table 3). There were larger and more numerous lesions in posterior ibotenic group, but only 6 with lesion scores corresponding to ~30+% cell loss, one of which was a unilateral hit (Table 4). Grouped data was done for posterior groups; only IBO-injected subjects with ~30+% bilateral cell loss (lesion score less than 0.7) were included in lesion group (n = 5). For anterior groups, since there were no subjects with ~30+% bilateral cell loss, we lowered lesion threshold to 20%, giving n=4. Nissl staining was not used for quantification of lesions. See "Materials and Methods" section for calculation of lesion score (1 = no lesion; 0 = complete lesion) and more detailed explanation of exclusion criteria. See "Appendix" for table with both scores for each animal.

3.2 Locomotor Data: Ambulatory Distance and Average Velocity

Spontaneous locomotor activity was assessed to ensure no differences existed between control and IBO-injected animals, which could affect performance in other behavioral tests. First we assessed the distance traveled during the first 5 minute block of testing on the first day; we did not use total day 1 distance in the event there were differences in short-term habituation to locomotor boxes.

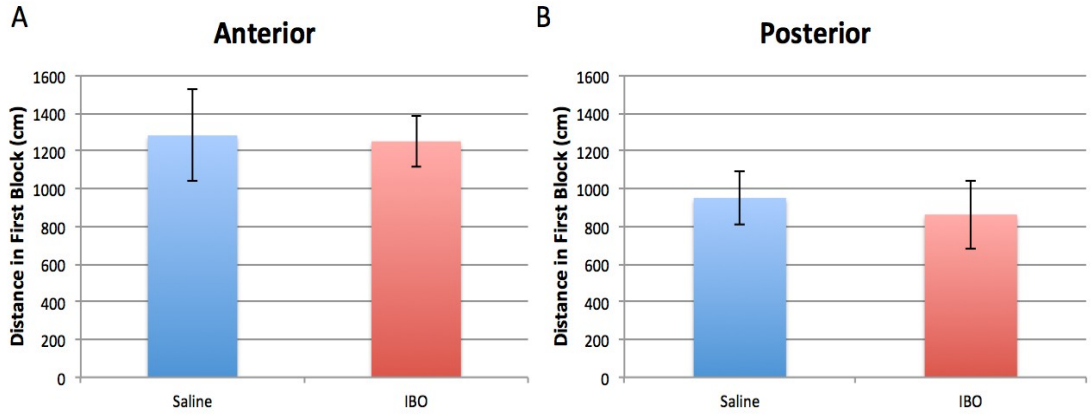

Figure 4: Ambulatory Distance (cm) during block 1 of day 1 of locomotor testing for (A) anterior and (B) posterior groups. There were no significant differences in distance traveled for either anterior (p=0.809) or posterior (p=0.399) groups, suggesting PPT cell loss does not affect spontaneous locomotion. AS: n=10; AI: n=4; PS: n=10; PI: n=4.

There were no significant differences in distance traveled during first block of testing for anterior ($F_{(1,12)}$=0.061, p=0.809) or posterior ($F_{(1,12)}$=0.399, p=0.765) groups. One posterior IBO animal with >30% cell loss was excluded from grouped locomotor data due to equipment failure during testing. Although there were no differences with grouped data, we next assessed correlations between lesion score and distance traveled since grouped data did not consider extent of lesion. Figure 5 shows correlations for anterior and posterior groups. There were no significant correlations between lesion score and distance traveled for anterior saline (r=-0.058, p=0.437), anterior

ibotenic (r= -0.442, p=0.065), posterior saline (r=0.389, p=0.267), or posterior ibotenic (r=-0.092, p=0.423) groups. Since there was significant overlap between saline and IBO groups, we also assessed overall correlation between lesion score and distance traveled by combining saline and IBO data. There were no significant correlations after doing this for anterior surgery groups (Fig 5A, r = -0.111, p=0.307) or posterior groups (Fig 5B, r=-0.225, p=0.193). These overall correlations are not shown with trend-lines in figures. These results suggest no effect on total distance traveled due to lesion score.

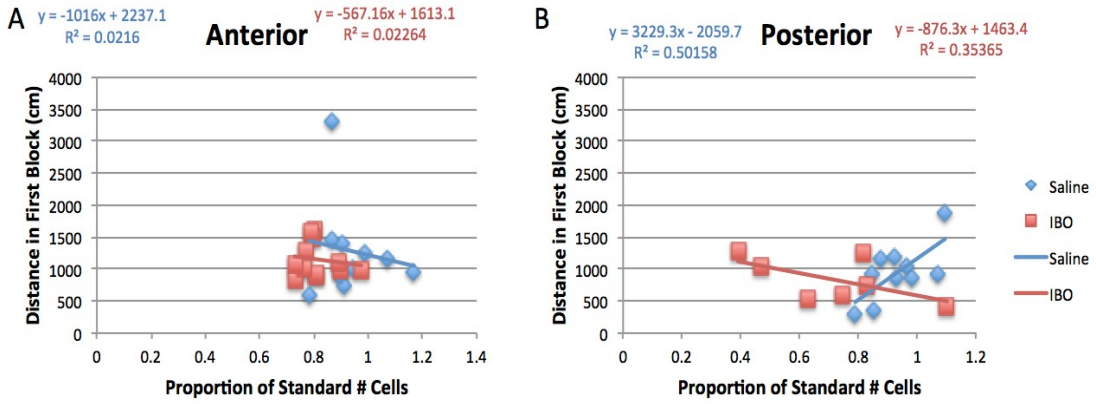

Figure 5: Ambulatory distance (cm) correlated to lesion score for (A) anterior and (B) posterior surgery groups. There were no significant correlations between lesion score and distance traveled for either anterior or posterior groups, suggesting PPT cell loss does not affect spontaneous locomotion. AS: n=10; AI: n=12; PS: n=10; PI: n=7.

We also assessed average velocity (Fig 6). We assessed average velocity only during the first block. There were no differences between anterior ($F_{(1,12)}$=0.368, p=0.556) or posterior ($F_{(1,12)}$=1.224, p=0.290) groups.

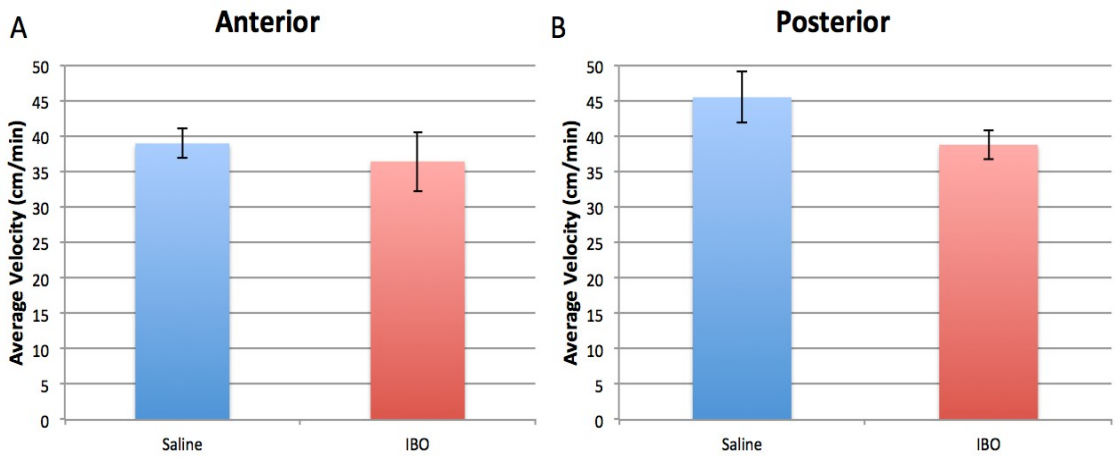

Figure 6: Average velocity (cm/min) during block 1 of day 1 of locomotor testing for (A) anterior and (B) posterior surgery groups. There were no significant differences in average velocity between anterior (p=0.556) or posterior (p=0.290) groups, suggesting PPT cell loss does not impair average velocity. AS: n=10; AI: n=4; PS: n=10; PI: n=4.

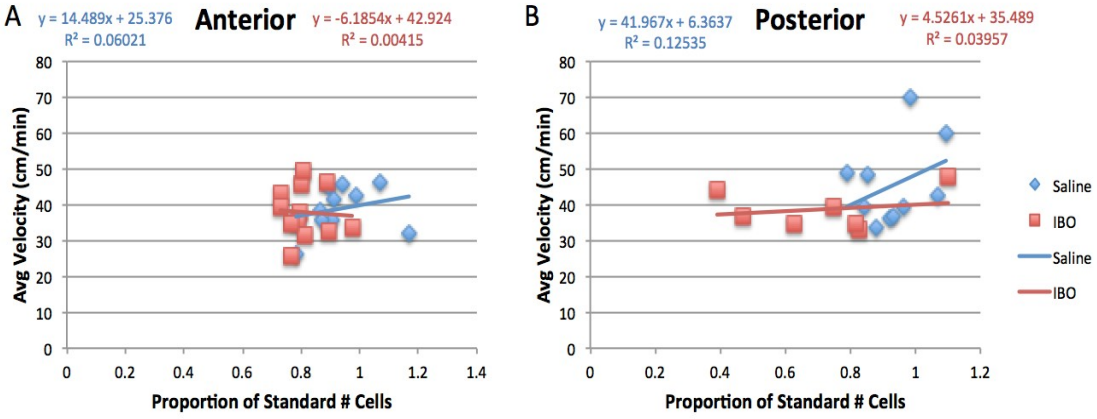

Figure 7: Average velocity (cm/min) correlated to lesion score for (A) anterior and (B) posterior surgery groups. There were no significant correlations between PPT cell loss and average velocity, suggesting PPT cell loss does not impair average velocity. AS: n=10; AI: n=12; PS: n=10; PI: n=7.

We also correlated average velocity to lesion score for anterior and posterior groups (Fig 7). Correlations between lesion score and average velocity were not significant for anterior saline (r=0.245, p=0.247), anterior IBO (r=-0.021, p=0.473), posterior saline (r=0.354, p=0.158), or posterior IBO (r=0.199, p=0.334) groups. Since there was

considerable overlap between groups, we determined overall correlations between lesion scores and behavioral data by combining saline and IBO data. There were no significant correlations in anterior ($r=0.147$, $p=0.252$) or posterior ($r=0.235$, $p=0.094$) groups after doing this. Lack of correlation between lesion score and average velocity suggests PPT cell count also does not affect average velocity.

In summary, there did not appear to be locomotor deficits associated with anterior or posterior PPT lesions, as assessed by total distance traveled and average velocity.

3.3 Startle Amplitude

Prior to analyzing habituation or prepulse inhibition data, startle values of each animal were tested to ensure no differences existed that could affect measurement of sensorimotor gating processes (Fig 8, 9). Fig 8 shows average initial startle amplitude by day while Fig 9 shows average habituated startle amplitude each day. Habituated startle amplitude is the baseline when assessing prepulse inhibition, and can be measured after short-term habituation processes have occurred.

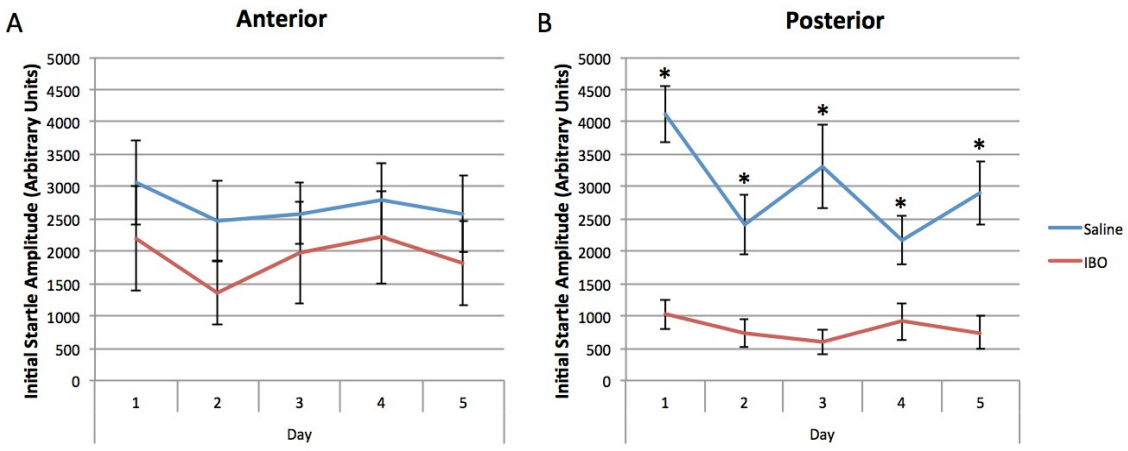

Figure 8: Initial startle amplitude by day for (A) anterior and (B) posterior groups. There were no differences in initial startle amplitude between anterior saline and IBO animals, but posterior IBO animals startled significantly lower than controls every day, suggesting cell loss in pPPT reduces startle amplitude ($p<0.001$). AS: n=10; AI: n=4; PS: n=10; PI: n=5.

There were no significant differences in initial startle amplitudes between anterior groups ($F_{(1,12)}=0.822$, $p=0.382$), but posterior IBO animals had significantly lower initial startle amplitudes than controls across all testing days ($F_{(1,13)}=21.196$, $p<0.001$). Similarly, there were no significant differences in baseline (habituated) startle amplitudes between anterior groups ($F_{(1,12)}=0.063$, $p=0.805$), but posterior IBO animals had significantly lower habituated startle amplitudes than controls across all testing days ($F_{(1,13)}=7.150$, $p=0.019$). These results suggest lesions of pPPT reduce amplitude of the startle response while aPPT lesions seem to have no effect.

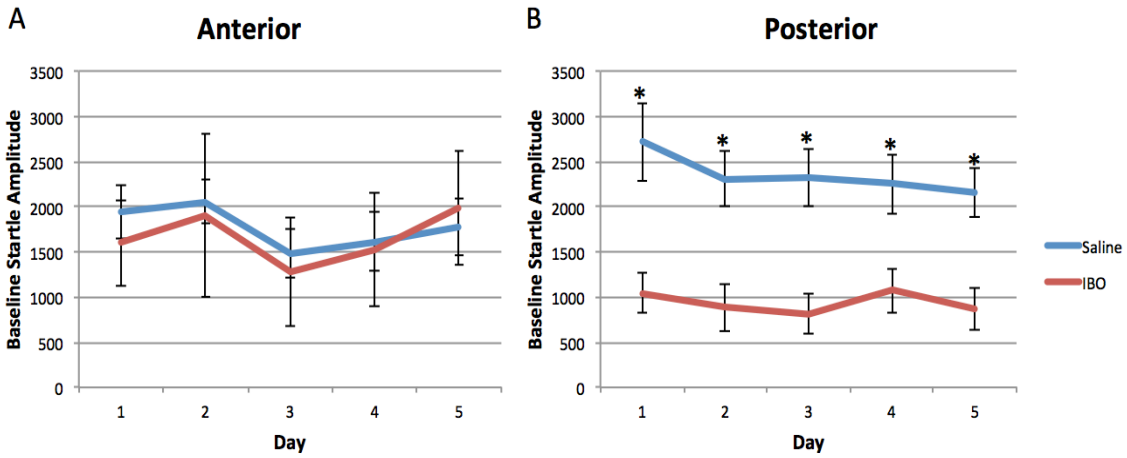

Figure 9: Baseline startle amplitudes by day for (A) anterior and (B) posterior groups. There were no differences in baseline startle amplitude between saline and IBO animals in anterior groups, but posterior IBO animals startled significantly lower than controls every day, suggesting cell loss in posterior PPT reduces startle amplitude ($p<0.001$). AS: n=10; AI: n=4; PS: n=10; PI: n=5.

We then assessed correlations between lesion score and initial (Fig 10) and habituated (Fig 11) startle amplitudes for anterior (A) and posterior (B) groups.

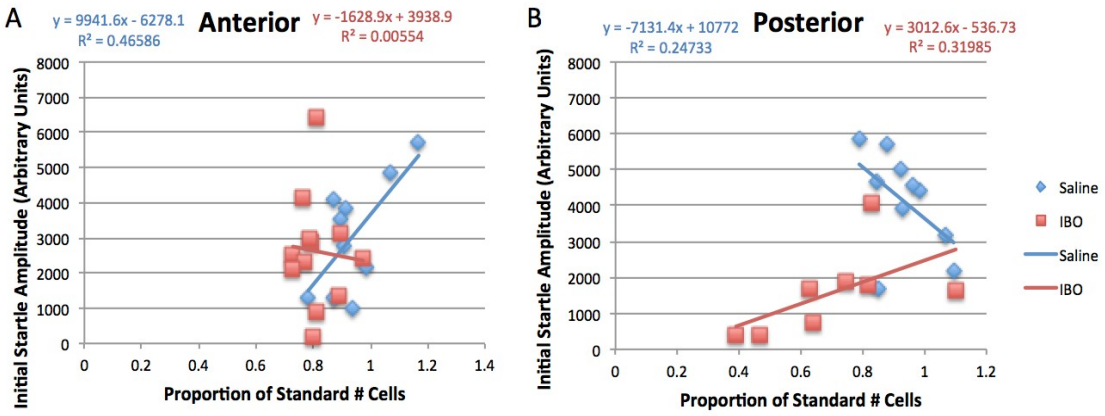

Figure 10: Initial startle amplitude (day 1) correlated to lesion score for (A) anterior and (B) posterior groups. Lesion score correlated with initial startle amplitude for anterior saline animals ($p=0.015$), and correlations between lesion score and initial amplitude for posterior groups approached significance. These results suggest a relationship between number of PPT cells and startle amplitude. AS: n=10; AI: n=12; PS: n=10; PI: n=8.

There was a significant correlation between lesion score and startle amplitude for anterior saline groups (r=0.683, p=0.015), but not for anterior IBO animals (r=-0.342, p=0.126). Although they approached significance, correlations between lesion score and initial startle amplitude were not significant for either posterior saline (r=-0.497, p=0.072) or posterior IBO (r=0.566, p=0.072) groups. Combining saline and IBO data, there was no significant correlation for anterior groups (p=0.298), but there was for posterior groups (r=0.496, p=0.018). Although correlations between lesion score and startle amplitude were not significant for posterior groups separately, they did approach significance; in addition, combined saline/IBO data was significantly correlated, and IBO animals had significantly lower startle amplitudes in grouped data. These results suggest pPPT lesion negatively impacts initial startle amplitude.

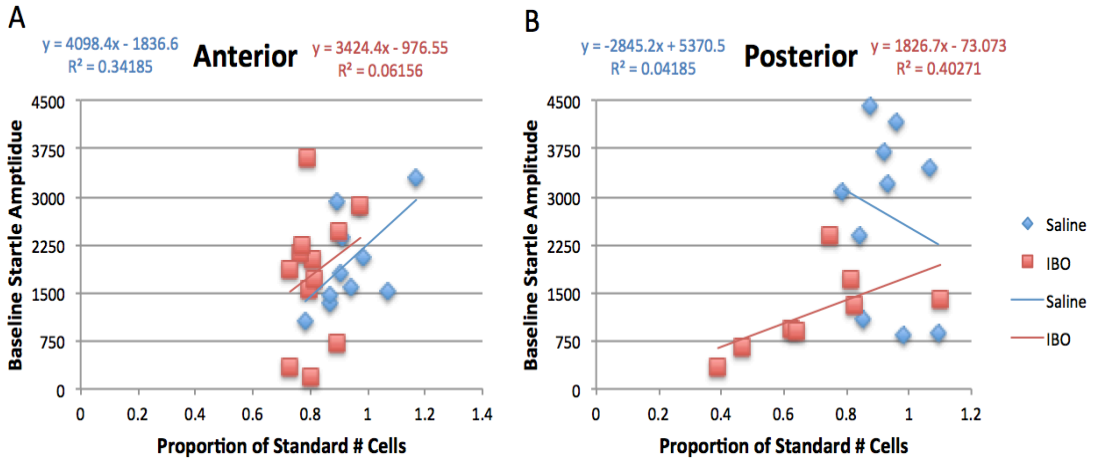

Figure 11: Baseline startle amplitude in pulse-alone (no prepulse) trials correlated to lesion score for (A) anterior and (B) posterior groups. There were no significant correlations between lesion score and baseline startle amplitude for anterior IBO or posterior saline groups, but there were for anterior saline and posterior IBO. These results further suggest a relationship between number of PPT neurons and startle amplitude. AS: n=10; AI: n=12; PS: n=10; PI: n=8.

Baseline startle amplitudes were not significantly correlated with lesion score for anterior IBO (r=0.225, p=0.23) or posterior saline (r=-0.183, p=0.613) groups, but lesion score was significantly correlated with baseline startle amplitude for anterior saline (r=0.467, p=0.03) and posterior IBO animals (r=0.635, p=0.045). Combining saline/IBO

data, there was a significant correlation between lesion score and baseline startle amplitude for posterior ($r=0.505$, $p=0.016$), but not anterior ($r=0.352$, $p=0.108$) groups.

In summary, these results suggest pPPT lesions reduce both initial and habituated startle amplitudes, which may affect measurement of habituation and prepulse inhibition.

3.4 Short-term Habituation

Assessed next was short-term habituation (STH) of the startle response (Fig 12). There were no significant differences in STH between saline and IBO animals in anterior groups ($F_{(1,12)}$=0.049, p=0.828), but differences between posterior groups approached significance ($F_{(1,13)}$=3.961, p=0.068). However, this data may be compounded by differences in startle amplitude.

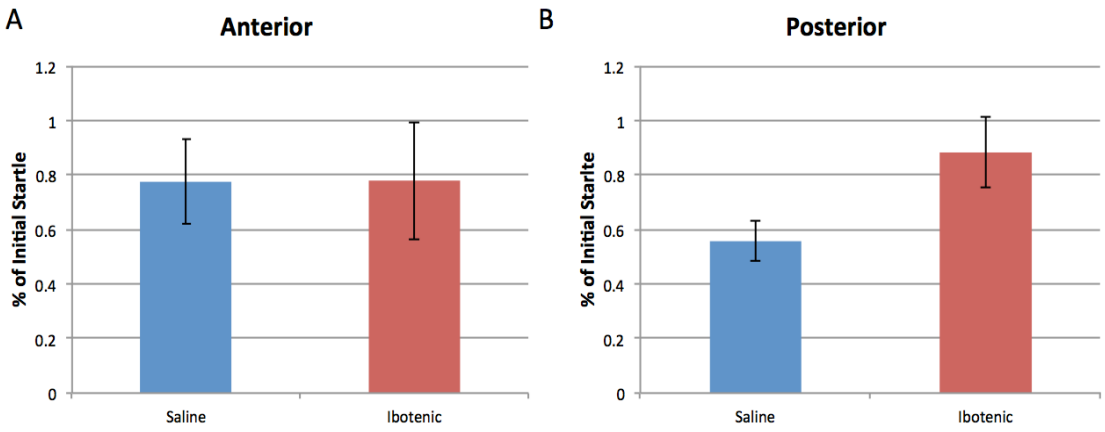

Figure 12: Short-term habituation (% of initial startle) for (A) anterior and (B) posterior groups. There were no significant differences in short-term habituation between saline and IBO animals in anterior groups, but differences in posterior groups approached significance. These results would suggest role for pPPT in STH, although differences in startle amplitude must be considered. AS: n=10; AI: n=4; PS: n=10; PI: n=5.

We also assessed correlations between lesion score and STH for saline and IBO groups (Fig 13). There were no significant correlations for anterior saline (r=-0.235, p=0.257); anterior IBO (r=0.344, p=0.125); posterior saline (r=0.297, p=0.203); or posterior IBO (r=-0.447, p=0.133) groups. Combining saline and ibotenic lesion scores and STH data, there were no significant correlations in anterior (r=0.059, p=0.394) or posterior (r=-0.15, p=0.192) groups.

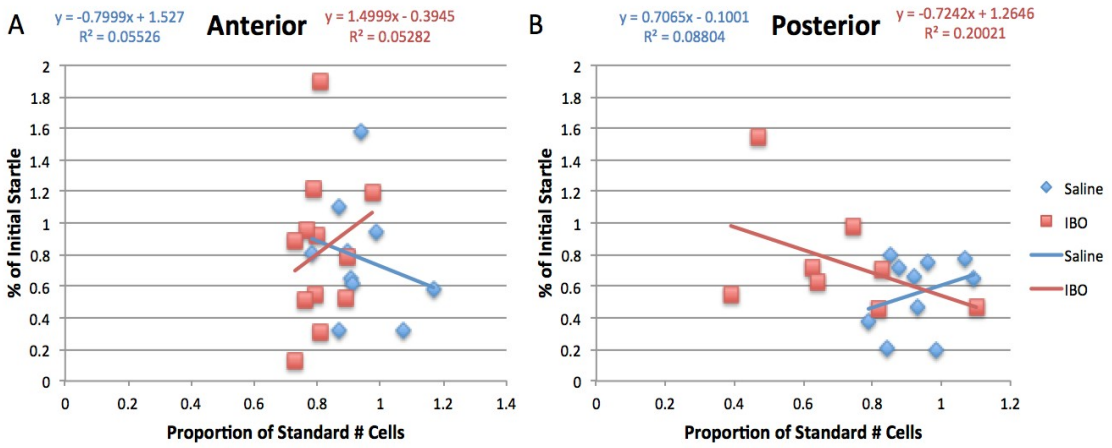

Figure 13: Short-term habituation (% of initial startle) correlated to lesion score for (A) anterior and (B) posterior groups. There were no significant correlations between lesion score and short-term habituation for anterior saline (p=0.257), anterior IBO (p=0.125), or posterior saline (p=0.203) groups, but correlation for posterior IBO approached significance (p=0.056). Differences in short-term habituation may arise from differences in startle amplitudes. AS: n=10; AI: n=12; PS: n=10; PI: n=8.

STH does not appear to be correlated with PPT integrity in either anterior or posterior animals. Although grouped posterior data approached significance, there was only five IBO animals included and results may have been confounded by the lower startle amplitude of posterior IBO animals, which would have less room to habituate. Together, these results do not suggest differences in STH between groups that could affect measurement of prepulse inhibition.

3.5 Long-term Habituation

Long-term habituation was assessed next (Fig 14) to probe whether PPT cell loss affected this phenomenon. Fig 14 shows initial startle amplitude of each day divided by the initial startle amplitude of day 1 to normalize the data and control for differences in startle amplitudes.

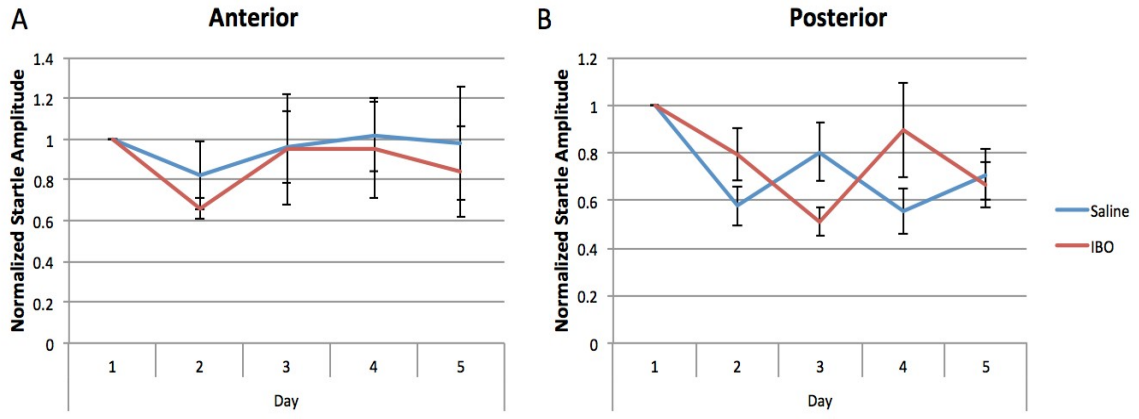

Figure 14: Initial startle amplitude each day (normalized to day 1) for (A) anterior and (B) posterior subjects. There were no significant differences in long-term habituation between saline and IBO animals in either anterior or posterior groups, suggesting neither anterior nor posterior PPT plays role in long-term habituation. AS: n=10; AI: n=4; PS: n=10; PI: n=5.

There were no significant differences in long-term habituation between saline and IBO animals in either anterior ($F_{(1,12)}$=0.155, p=0.700) or posterior ($F_{(1,13)}$=0.183, p=0.676) groups. We then correlated lesion score to LTH score (Day 5/Day 1).

There were no significant correlations between lesion scores and LTH scores in anterior saline (r=-0.025, p=0.473); posterior saline (r=0.26, p=0.234); or posterior IBO (r=0.483, p=0.079) groups (Fig 15). Since anterior IBO data was not normally distributed, a non- parametric assessment of correlation was done; Spearman's coefficient for this correlation was 0.132, with p=0.334. There were no significant correlations for combined saline/IBO data for either anterior (r=0.154, p=0.241) or posterior (r=0.321, p=0.097) groups.

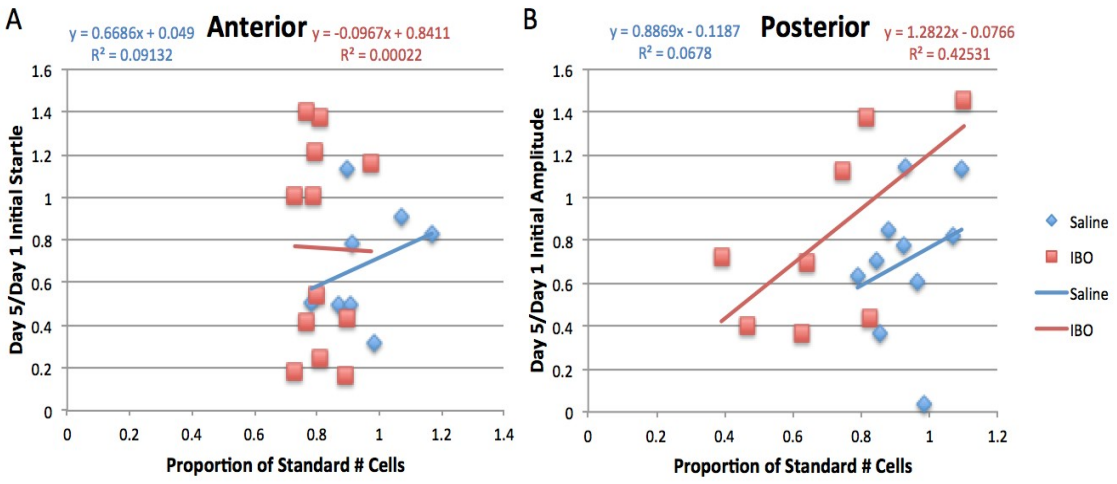

Figure 15: Long-term habituation (day5/day 1 initial startle) correlated to lesion scores for (A) anterior and (B) posterior groups. There were no significant correlations between lesion score and LTH. While correlation for posterior IBO group approached significance, differences in startle amplitude must be considered. AS: n=10; AI: n=12; PS: n=10; PI: n=8.

Together, these results suggest that, despite differences in startle amplitude in posterior groups, long-term habituation processes are not disrupted by PPT cell death, consistent with expectations. The apparent bimodal distribution of LTH scores for anterior IBO animals is largely explained by differences in startle amplitude. Correlation between initial startle amplitude and LTH score approached significance (r=-0.394, p=0.092). Thus, it appears the bimodal distribution may arise from differences in startle amplitude.

3.6 Prepulse Inhibition

Figure 16 displays prepulse inhibition (% inhibition) at two prepulse levels (75dB, 85dB) and three interstimulus intervals (10ms, 30ms, 100ms). There were no significant differences in % inhibition at either prepulse for any interstimulus interval tested for anterior groups ($F_{(1,12)}$=1.811, p=0.203) or posterior groups ($F_{(1,13)}$=1.346, p=0.267).

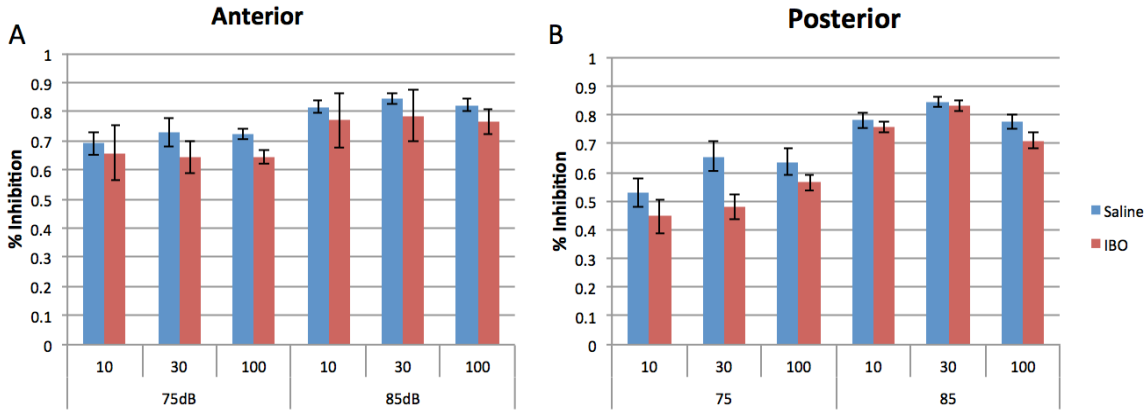

Figure 16: Prepulse inhibition (% inhibition) for (A) anterior and (B) posterior groups with 75 & 85dB prepulses at 10, 30, and 100ms prior to 105dB startle stimulus. There were no significant differences between saline and IBO animals for either anterior (p=0.203) or posterior (p=0.267) groups for the parameters tested. These results do not support PPT role in PPI. AS: n=10; AI: n=4; PS: n=10; PI: n=5.

Differences in PPI between control and IBO animals at 75dB prepulse approach significance at 30ms ISIs ($F_{(2,12)}$=3.815, p=0.052). Since grouping animals masks variability in behavior and does not account for variability in extent of lesion, correlations were done between lesion scores and prepulse inhibition at 10 and 30ms ISIs and 75dB prepulse. Lack of differences at 85dB suggests relatively mild PPI deficits that are only detected with relatively less neural activation at 75dB prepulse. PPI data is presented for anterior (Fig 17) and posterior (Fig 18) animals.

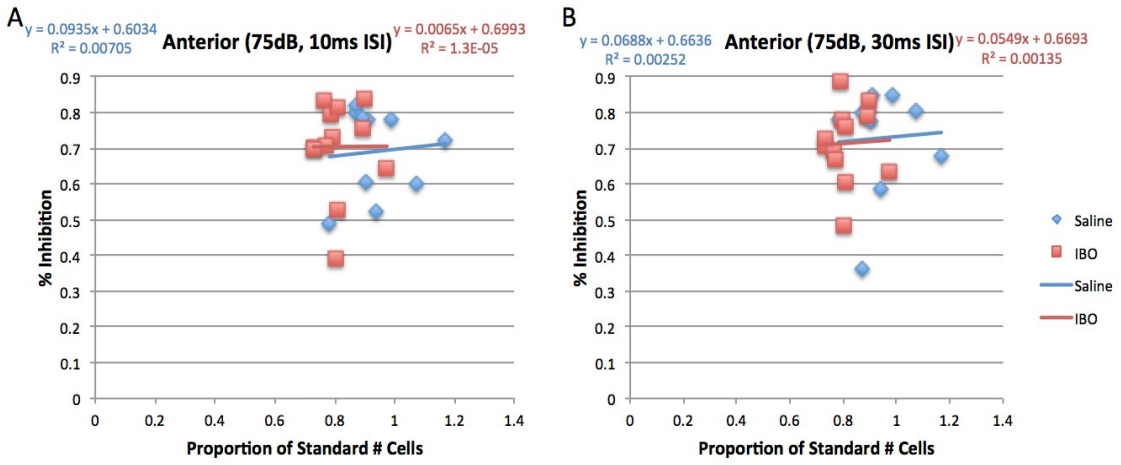

Figure 17: Prepulse inhibition (% inhibition) for anterior groups with 75dB prepulse at (A) 10ms and (B) 30ms ISI. There were no significant correlations between lesion score and PPI for anterior saline groups at 10ms (p=0.409) or 30ms (p=0.445) or for anterior IBO groups at 10ms (p=0.327) or 30ms (p=0.094) ISI. These results suggest cell loss in anterior PPT does not impair PPI. AS: n=10; AI: n=12.

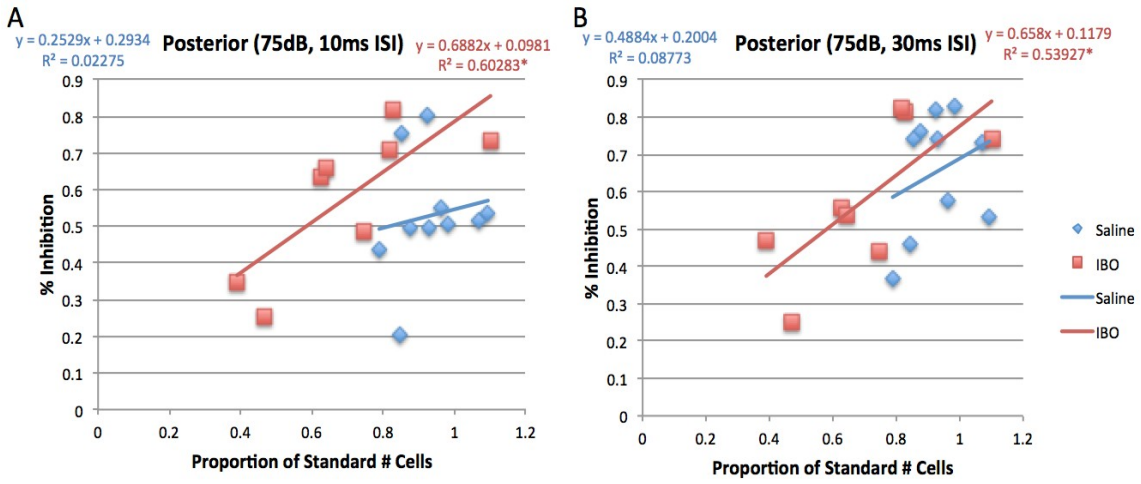

Figure 18: Prepulse inhibition (% inhibition) for posterior groups with 75dB prepulse at (A) 10ms and (B) 30ms ISI. There were no significant correlations for posterior saline groups at either ISI. Correlations between lesion score and PPI were significant at both 10ms (p=0.012) and 30ms (p=0.019) ISI, suggesting cell loss in pPPT weakens PPI. PS: n=10; PI: n=8.

There were no significant correlations between lesion score and % inhibition for anterior saline animals at 10ms (r=0.084, p=0.409) or 30ms (r=0.05, p=0.445) ISI; similarly, there were no significant correlations for anterior IBO animals at 10ms (r=0.137, p=0.327) or 30ms (r=0.39, p=0.094). Combining saline and IBO data, there was also no significant correlation between lesion score and % inhibition for anterior groups at either 10ms (r=0.08, p=0.358) or 30ms (r=0.102, p=0.275).

There were no significant correlations between lesion score and % inhibition for posterior saline animals at 10ms (r=0.151, p=0.339) or 30ms (r=0.296, p=0.203) ISI. Conversely, lesion score was significantly correlated to PPI in posterior IBO animals at 75dB prepulse at both 10ms (r=0.776, p=0.012) and 30ms (r=0.724, p=0.019) ISIs. The correlation between lesion score and % inhibition with combined saline/IBO data was nearly significant (r=0.352, p=0.076). These results suggest a relationship between pPPT integrity and prepulse inhibition processes.

3.7 Morris Water Maze

Lastly, the cued and spatial Morris water maze data was analyzed. Both distance (m) and latency to target (s) were assessed, but since they were very similar, only distance to target data is shown.

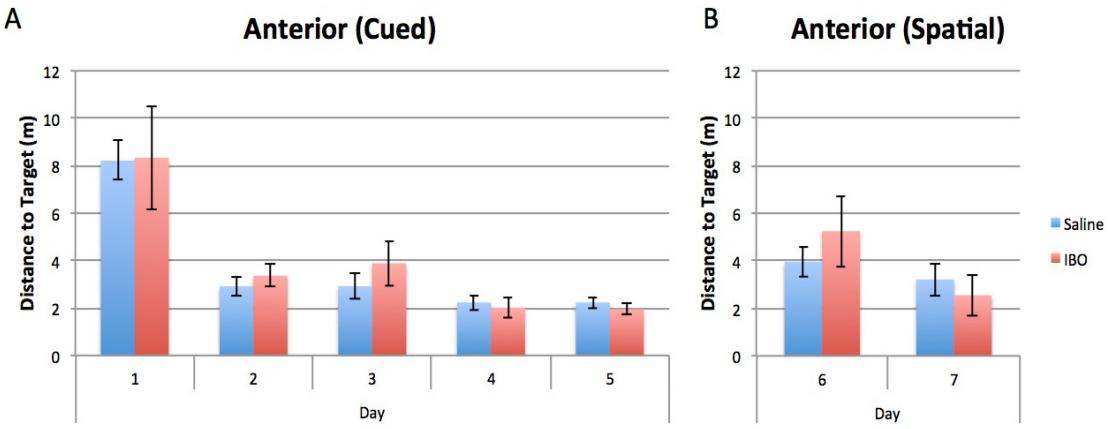

Figure 19: Distance to target (m) by day for anterior groups in (A) cued and (B) spatial tasks. There were no significant differences between saline and IBO animals in either cued (p=0.884) or spatial (p=0.717) tasks, suggesting anterior PPT cell loss does not impair performance in either task. AS: n=10; AI: n=4.

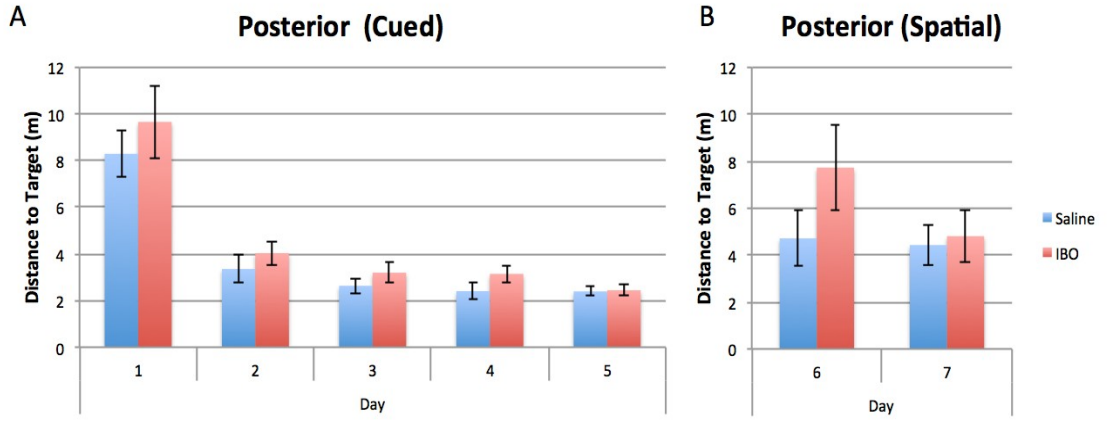

Figure 20: Distance to target (m) by day for posterior groups in (A) cued and (B) spatial tasks. There were no significant differences between saline and IBO animals in either cued (p=0.151) or spatial (p=0.857) tasks, suggesting posterior PPT cell loss does not impair performance in either task. PS: n=10; PI: n=5.

There were no differences in distance to target for anterior groups for either cued ($F_{(1,12)}$=0.022, p=0.884) or spatial ($F_{(1,13)}$=0.138, p=0.717) tasks (Fig 19). There were also no differences in distance to target for posterior groups in cued ($F_{(1,13)}$=2.328, p=0.151) or spatial ($F_{(1,13)}$=0.034, p=0.857) tasks (Fig 20). Although there were no differences by day, most learning occurred on day 1. We assessed day 1 trials for both anterior (Fig 21) and posterior (Fig 22) groups.

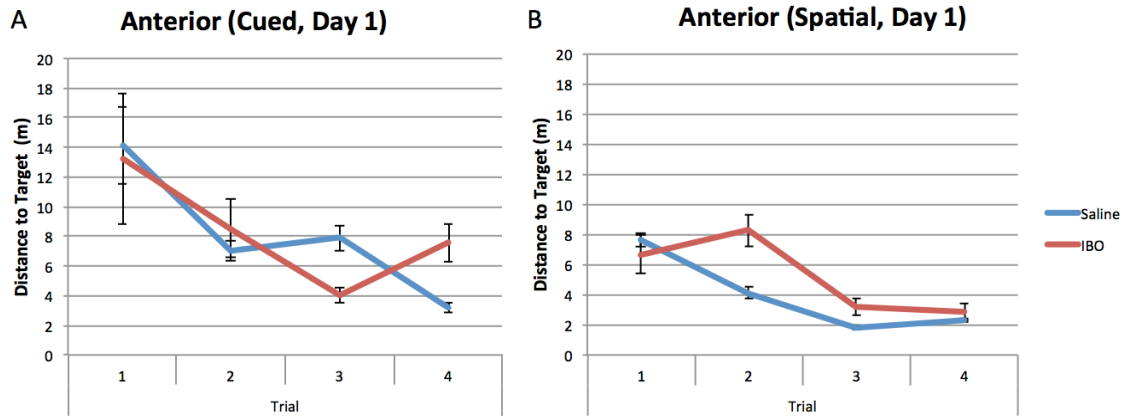

Figure 21: Distance to target (m) by trial for anterior groups on day 1 of (A) cued and (B) spatial tasks. There were no differences between saline and IBO animals either cued (p=0.885) or spatial (p=0.358) task, suggesting lack of aPPT role in these forms of learning. AS: n=10; AI: n=4.

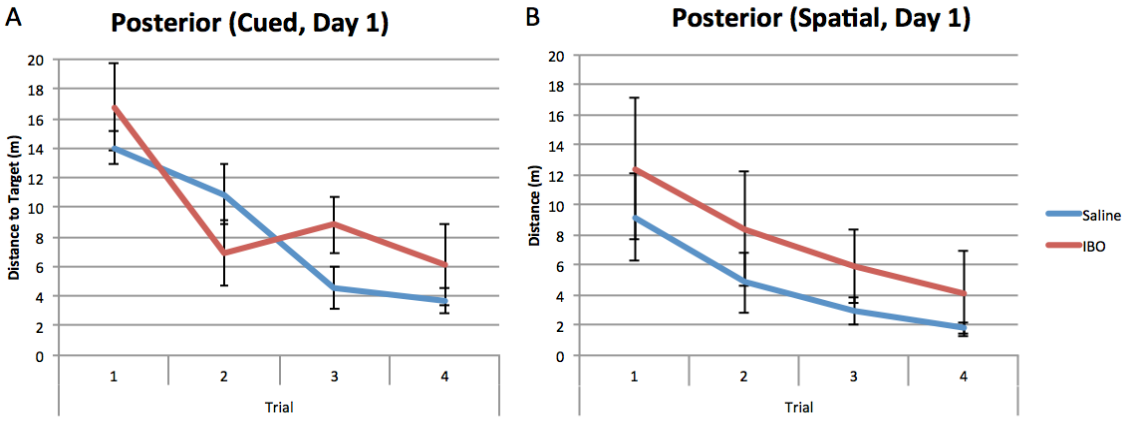

Figure 22: Distance to target (m) by trial for posterior groups on day 1 of (A) cued and (B) spatial tasks. There were no differences between saline and IBO animals either cued (p=0.133) or spatial (p=0.909) task, suggesting lack of pPPT role in these forms of learning. PS: n=10; PI: n=5.

There were no significant differences in distance to target for anterior groups on day 1 of either cued ($F_{(1,12)}$=0.022, p=0.885) or spatial ($F_{(1,12)}$=0.914, p=0.358) tasks. There were also no significant differences in distance to target on day 1 for posterior groups in either cued ($F_{(1,13)}$=2.567, p=0.133) or spatial ($F_{(1,13)}$=0.014, p=0.909) tasks. These data together suggest there are no differences in performance in either water maze task following PPT cell loss.

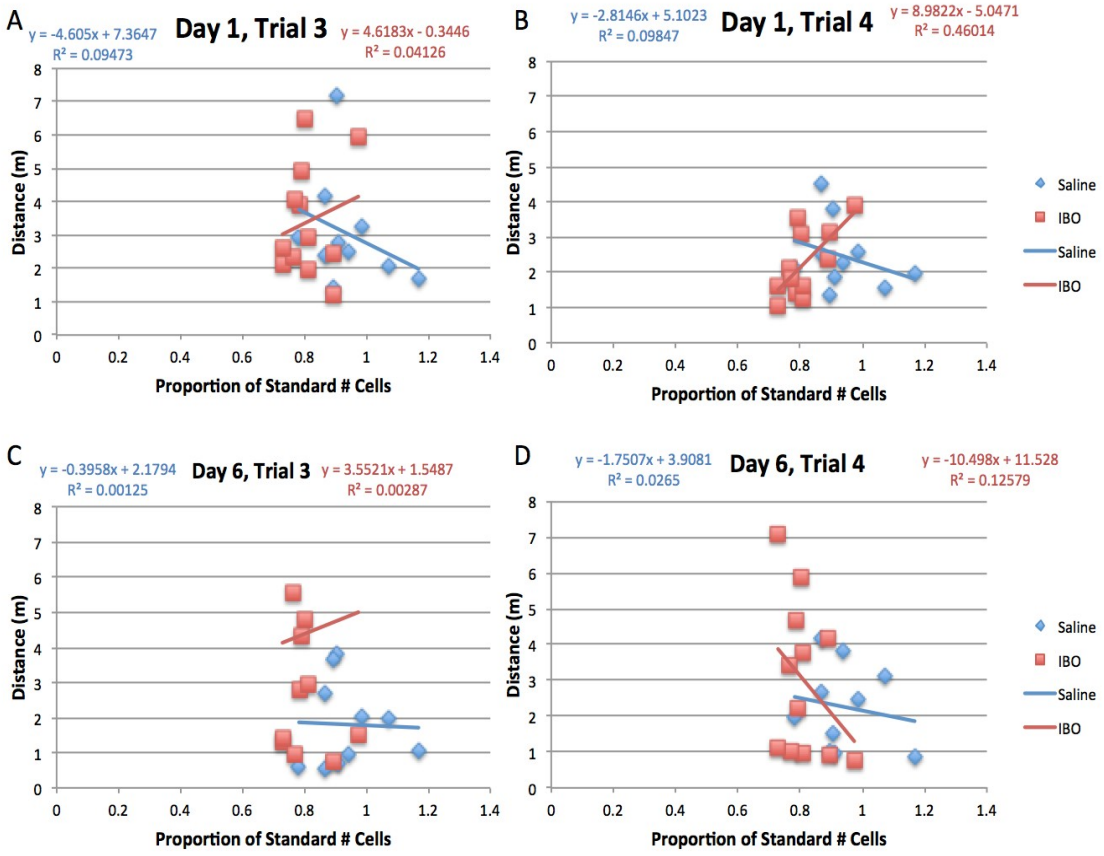

Figure 23: Distance to target (m) for anterior groups in trials 3 and 4 on day 1 for (A,B) cued and (C,D) spatial tasks. The only significant correlation was for anterior IBO animals on day 1, trial 4, although this correlation would suggest cell loss improves performance in the cued task. These results fail to support aPPT role in either task. AS: n=10; AI: n=12.

Lesion scores were then correlated to behavioral data for trials 3 and 4 of days 1 and 6 – trials with the most learning. Day 6 is day 1 of the spatial task. Fig 23 shows distance to

target (m) for anterior groups for these four different trials. Fig 24 shows distance to target (m) for posterior groups for these same four trials (trials 3 and 4 of days 1 and 6).

There were no significant correlations for anterior saline animals on day 1, trial 3 (r=-0.308, p=0.193); day 1, trial 4 (r=-0.314, p=0.189); day 6, trial 3 (r=-0.035, p=0.461); or day 6, trial 4 (r=-0.163, p=0.327). There were no significant correlations for anterior IBO animals on day 1, trial 3 (r=0.176, p=0.283); day 6, trial 3 (r=0.133, p=0.333); or day 6, trial 4 (r=-0.227, p=0.228). However, there was a significant correlation between lesion score and distance to target on day 1, trial 4 for anterior IBO animals (r=0.611, p=0.013). However, an overall correlation between lesion score and distance to target on day 1, trial 4 for anterior animals after combining saline and IBO data is not significant (r=0.231, p=0.161). There is also considerable overlap between distance to target scores on day 1, trial 4 for anterior saline and IBO animals. These results do not support a relationship between aPPT and performance in cued or spatial version of Morris water maze.

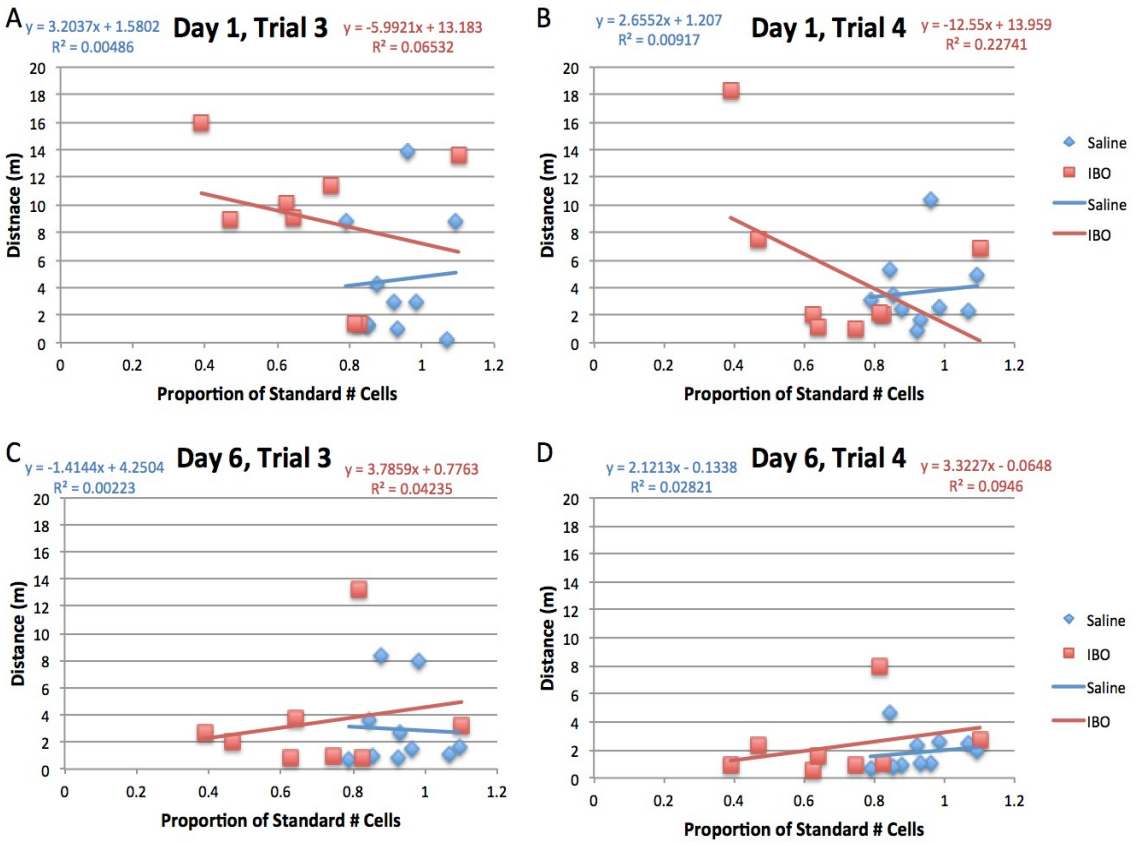

Figure 24: Distance to target (m) for posterior groups on trials 3 and 4 on day 1 of (A,B) cued and (C,D) spatial tasks. There were no significant correlations between lesion score and distance to target for either saline or IBO posterior animals. These results fail to support a relationship between cell loss in pPPT and performance in these tasks. PS: n=10; PI: n=8.

There were no significant correlations for any of the four trials analyzed for posterior saline animals: day 1, trial 3 (r=-0.181, p=0.308), day 1, trial 4 (r=-0.462, p=0.09), day 6, trial 3 (r=-0.017, p=0.481), or day 6, trial 4 (r=0.153, p=0.336). For posterior IBO animals, there were no significant correlations between lesion score and distance to target for day 1, trial 3 (r=-0.256, p=0.271); day 1, trial 4 (r=-0.477, p=0.116); day 6, trial 3 (r=0.206, p=0.312) or day 6, trial 4 (r=0.308, p=0.229). These results indicate no relationship between pPPT and performance in these trials of Morris water maze, consistent with expectations that pPPT not important for either cued or spatial version of water maze task.

Chapter 4

4 Discussion

Ibotenic acid lesions of anterior PPT caused no obvious deficits, although no subjects exceeded 30% lesion threshold. Conversely, IBO lesions of posterior PPT reduced startle amplitude, and extent of PPT lesion was negatively correlated to prepulse inhibition.

4.1 Methodological Issues

Lesion Surgery: Selection and Concentration of Lesioning Agent

Lesion studies often face criticism due to unintended damage inflicted by lesioning agent outside target area. While conventional lesion techniques (aspiration, electrolytic, radiofrequency) have been replaced by more selective methods, such as excitotoxins, there is still no way to ensure damage is limited to area of interest (Jarrard, 2002). Interpretation of results of any lesion study should consider this limitation.

Ibotenic acid is a potent excitotoxin that acts on NMDA glutamate receptors, and although it spares fibers passing through target area, it is not selective for any particular cell type, and thus injection of ibotenic acid into PPT also causes significant non-cholinergic cell death (Dunbar et al., 1992). Although quinolinic acid is an excitotoxin more selective for cholinergic neurons than ibotenic acid (Dunbar et al. 1992), we used ibotenic acid for two main reasons:

1) Lesion studies discussed in introduction also used ibotenic acid as lesioning agent (except for Koch et al., 1993), so its use in this study maintained consistency with other groups in the field.
2) Our lab obtained a very limited quantity of a novel lesioning tool (diphtheria toxin fused to Urotensin II) that is highly selective for mesopontine

cholinergic cells (PPT, LDT), which are only cells in region to express Urotensin II receptor (Clark et al., 2005; Nothacker and Clark, 2005; Clark et al., 2007). Original intent was to use ibotenic acid to identify deficits from aPPT/pPPT lesions, then this novel toxin to identify which were specifically cholinergic.

In this study, 400nL 0.063M ibotenic acid (IBO) was injected into PPT on each side of brain. While Winn group uses 200nL 0.12M IBO (Alderson et al., 2002; Alderson et al., 2004; Alderson et al., 2006; Wilson et al., 2009; MacLaren et al., 2013), other studies have effectively used much lower concentrations, including PPT lesion studies (Jarrard, 1989; Deurveilher and Hennevin, 2001; Florio et al., 2001). Although every effort was made to reach concentration of 0.12M IBO, including use of same vehicle as Winn group (phosphate buffer, pH 7.4), maximum achievable concentration was 0.063M (10mg/ml). To deliver same dose as Winn group (24nmol), twice the volume was injected. Although this does increase risk of spreading dose over a greater area, there was greater risk of insufficient lesions at 200nL, as lower doses inflict much less damage (Jarrard, 2002).

Compensation after PPT lesion

Bilateral PPT surgery in rats in this surgery had a high mortality rate, consistent with other studies (Alderson et al., 2002; Winn, 2006, 2008). Separating left and right PPT IBO injections by one week eliminated any further animal deaths from surgery. One week is the typical separation between left and right PPT lesions by other groups (Alderson et al., 2002; Alderson et al., 2004; Alderson et al., 2006; Wilson et al., 2009; MacLaren et al., 2013). Reduction in mortality rate after separation of right and left lesions is indicative of compensation for PPT cell loss. Compensation may mask deficits in functions that would otherwise be attributed to PPT.

NADPH and Nissl Staining

Verifying and quantifying lesions was essential for proper interpretation of

behavioral data; correlating extent of lesion with performance in various paradigms provided greater insight into relationship between PPT and function in question. NADPH staining was done to visualize cholinergic cells and Nissl staining was done to visualize all cells. NADPH specificity for cholinergic neurons enabled manual counting of cells and thus, quantification of extent of lesion. Nissl staining was done to visualize non-cholinergic cell death, which stains all cells. Consequently, manual counting was not possible for Nissl stains. Estimates of lesion extent are thus based on NADPH histological analysis. Since cholinergic cells of PPT are typically more resistant to IBO than non-cholinergic cells (Dunbar et al., 1992), basing lesion score on NADPH staining is conservative and may underestimate extent of lesion.

Most groups consider PPT lesioned when cell loss exceeds 50% (Alderson et al., 2002; Wilson et al., 2009; MacLaren et al., 2013). While some cell loss was evident in most IBO subjects, especially in posterior groups, the data could not clearly be divided into lesioned and non-lesioned animals. Consequently, correlations were done to match behavioral data with integrity of PPT. However, lack of positive results – especially for anterior group – may be due to insufficient PPT cell death. Correlations address this issue to an extent, but it must be considered when interpreting data that lesions were not as extensive as typically required for deficits.

Morris Water Maze

This paradigm included 5 days of cued version of Morris water maze followed by two days of spatial, but was not balanced with 5 days of spatial followed by two days of cued. This is in contrast to other studies that did balance these tests. However, these studies were generally investigating the dissociation of allocentric and egocentric learning (Packard and McGaugh, 1992; 1996; Lee et al., 2008; Braun et al., 2012). Our experimental hypothesis pertained to dorsal striatal learning and cued version of Morris water maze; spatial task was done as a control to assess whether animals were deficient in spatial navigation and memory in general. No deficits were expected in spatial version of task in either anterior or posterior group.

Analysis of water maze performance focused on trials 3 and 4 of days 1 (cued) and 6 (spatial), and trial 1 of days 2 and 7 (latter results not shown). The PPT is important for acquisition of the associative memory, and these early trials probed acquisition of the cue-platform association. Most learning occurred during these early trials. Day 1, trial 3 was the first trial the association in cued version could be formed. It takes at least two trials to confirm rule that cue is associated with platform.

4.2 Locomotor Activity

Neither anterior nor posterior groups showed differences in locomotion between control and IBO animals. Rats in anterior group typically traveled 20-50m in their first session at approximately 40cm/min; similarly, rats in posterior group typically traveled 15-35m in their first session at roughly the same speed. While the PPT is important for locomotion in humans and primates and results in akinesia if lesioned (Munro-Davies et al., 2001), it appears less critical in rats, in agreement with other studies (Wilson et al., 2009; MacLaren et al., 2013). This supports the evidence that there are some functional differences between human and rat PPT, creating limitations when extrapolating experimental data from rats to humans. There were no correlations between lesion scores and locomotor data, suggesting PPT is not important for spontaneous locomotion in rats. These results confirm limitations of using a rat model to study PPT role in locomotion. In addition, these results confirm that other behavioral tests of this study were not confounded by presence of locomotor deficits.

4.3 Amplitude and Habituation of the Startle Response

There were no significant differences in startle amplitude between anterior groups. Conversely, both initial and habituated startle amplitudes of pPPT IBO animals were significantly lower than controls and were significantly correlated to lesion score. This

suggests pPPT lesions reduce amplitude of the startle response, in contrast to a previous study showing electrolytic PPT lesions potentiated startle amplitude (Swerdlow & Geyer 1993). However, electrolytic lesions could cause unintended damage to fibers passing through the PPT, and are less ideal than use of excitotoxins as a lesioning agent. Although expressing PPI as a percentage inhibition mitigates these differences in startle amplitudes to an extent, these disparities in startle may affect measurement of PPI. With that said, there was no correlation between baseline startle amplitude and PPI, indicating PPI score not dependent upon baseline startle amplitude (R^2=0.06).

Differences in startle amplitudes were not due to differences in weight. They were also not due to deficits in hearing: intact prepulse inhibition indicates ability to hear prepulse. One possibility is that, as part of reticular activating system, tonic ACh release in PnC maintains degree of excitability of giant neurons, similar to how cholinergic PPT input to thalamus increases excitability of thalamocortical relay neurons to incoming stimuli. Loss of this tonic input could decrease excitability of the startle-mediating neurons in PnC, making it harder for them to reach threshold. Fewer startle-mediating neurons reaching threshold would result in weaker startle response. A toxin more selective for cholinergic cells could determine whether this effect is due to loss of ACh input. In addition, control rats to the posterior lesion group were high startlers, with over half startling near or above 6000 arbitrary units (in contrast to anterior control rats). Since animals were not tested in startle boxes prior to surgery, it is unclear whether posterior IBO animals were also higher startlers before surgery and thus whether pPPT lesions really reduced startle amplitude or whether this effect is due to very unfortunate grouping.

There were no differences in short-term habituation scores between control and IBO-injected animals in anterior group, and there were no significant correlations between lesion scores and STH scores for anterior group. In posterior group, IBO animals showed weaker short-term habituation scores than controls. However, these animals also showed significantly lower initial and habituated startle amplitudes than control animals, which could influence measurement of STH since startle amplitudes of posterior IBO

animals would have less room to habituate. Furthermore, pPPT IBO-injected animals generally habituated to 60% of their initial response – similar to rats from anterior groups, while pPPT saline animals habituated further than this. These stronger habituation scores in control group could result from higher startle amplitudes having more room for habituation to occur.

There were also no significant differences or correlations between lesion scores and long-term habituation (LTH) scores for anterior groups. While there was a weak positive correlation between lesion scores and LTH scores for posterior groups, there were no significant LTH differences between control and IBO-injected animals. Similar to STH data, these higher LTH scores for posterior control groups could be due to high initial startle amplitude on day 1. Differences in initial startle amplitude confound interpretation in LTH data. These results suggest that there is no effect of aPPT lesions on startle amplitude or habituation (short- or long-term) processes. In contrast, pPPT lesions significantly reduce startle amplitude, which may have affected comparison of short- and long-term habituation.

4.4 Prepulse Inhibition of the Startle Response

Previous literature implicates both the PPT and acetylcholine in PPI (Koch et al., 1993; Swerdlow and Geyer, 1993; Fendt et al., 2001; Bosch and Schmid, 2008; Yeomans et al., 2010). Full PPT lesions impair PPI of the acoustic startle reflex in rats (Swerdlow & Geyer 1993; Koch et al., 1993). Furthermore, prepulse inhibition of the acoustic startle response is reduced at long interstimulus intervals (100-1000ms) by pharmacological inhibition of muscarinic or $GABA_B$ receptors (Bosch & Schmid 2008; Yeomans et al., 2010), suggesting acetylcholine and GABA mediate PPI at long interstimulus intervals.

Activation of $GABA_A$ receptors hyperpolarizes PnC cells (Yeomans et al., 2010), and nicotine can rescue methamphetamine-induced disruptions in PPI at 100ms ISI (Mizoguchi et al., 2009), suggesting a nicotinic and GABAergic role in PPI at shorter ISIs. These results suggest a cholinergic/GABAergic PPT role in mediating

PPI. Since pPPT receives fast collicular sensory input and sends descending projections to the PnC, we expected to find PPI deficits in rats with posterior but not anterior PPT lesions. There were no correlations between lesion scores and prepulse inhibition scores for anterior groups at either interstimulus interval (ISI) shown. PPI at 75dB prepulse with 10ms or 30ms ISI should be least robust relative to other combinations tested, and thus most sensitive to deficits. Lack of any correlation or differences for anterior groups suggests either aPPT is not involved in PPI, or not enough cell loss occurred to manifest PPI deficits.

Conversely, lesion scores and PPI scores for posterior groups were significantly correlated. Greater cholinergic cell loss was significantly correlated with lower PPI at 75dB, 10/30ms ISI. Although differences in baseline startle amplitude between posterior control and IBO animals could influence measurement of prepulse inhibition, there was no correlation between baseline startle amplitude and PPI score. Since PPI is also expressed as a percentage inhibition of baseline startle, differences in startle amplitude would be mitigated further. These results are consistent with our hypothesis that posterior, but not anterior, regions of PPT are important in PPI process and circuitry. However, these results do not specifically implicate cholinergic cells in PPI as GABAergic cell loss in PPT could also manifest as PPI deficits; thus, a toxin more selective for cholinergic cells, such as the diphtheria-Urotensin II toxin, could determine whether weaker PPI was due to loss of cholinergic cells and thus ACh input to PnC.

In summary, unless there was not enough cell loss in aPPT to cause PPI deficits, these results suggest that PPI sensorimotor gating function of PPT is localized to posterior regions that have projections downstream to PnC. Understanding the sensorimotor gating role of PPT provides insight into how higher brain regions modulate this process; although it is a basic process, prepulse inhibition is a building block of higher attentional processes given its importance in attention, and therefore PPI deficits may impair higher cognition (Graham, 1975; Braff et al., 1978; Ellenbroek, 2004; Giakoumaki, 2012; Kohl et al., 2013; Park et al., 2013).

4.5 Morris Water Maze

The cued version of the Morris water maze depends on action-outcome (action: swim to cue; outcome: reach platform) associative learning, dependent on dorsal striatum (Packard and McGaugh, 1992; McDonald and White, 1994; Packard and McGaugh, 1996; Thullier et al., 1996; Brasted et al., 1997; Alderson et al., 2002; Gengler et al., 2005; Lee et al., 2008). The PPT is important for acquisition of associations; rats with PPT lesions could not form association between lever pressing and food reward, but if association was learned prior to lesion surgery, they performed similar to controls (Alderson et al., 2004). Cholinergic modulation of VTA/SNc DAergic cells is believed to underlie this PPT role in reinforcement learning. While much evidence exists for pPPT role in acquisition of stimulus-reward associations (Alderson et al., 2006; Wilson et al., 2009; MacLaren et al., 2013), there is less support for an aPPT role in action-outcome learning. We expected aPPT-lesioned animals to show impaired performance in cued version of Morris water maze, while pPPT-lesioned animals were not expected to show deficits. Specifically, we expected early trials during acquisition of association to show the greatest differences in performance between control and aPPT-lesioned animals. We did not expect deficits in spatial water maze performance in either anterior or posterior groups. There were no correlations or differences for anterior groups in cued or spatial versions of Morris water maze. Although deficits were expected in anterior IBO-injected rats, it is likely that cell loss was insufficient to have observable behavioral deficits. These lesions are not sufficiently extensive to rule out an aPPT role in dorsal striatal reinforcement learning. In agreement with expectations, there were no deficits on day 6 (first day of spatial), with aPPT IBO-injected animals.

We did not expect pPPT-lesioned rats to show impairments in the cued version of the Morris water maze since projections to SNc DAergic cells arise largely from aPPT. Rats with dorsal striatal lesions were not impaired in performance of the spatial task (Packard and McGaugh, 1992, 1996; Thullier et al., 1996). In line with expectations, there were no correlations or differences among posterior groups on either day 1 or day

6. However, while improvement in performance between trials 3 and 4 for most rats is evident, rats with the most extensive pPPT lesions showed worst performance and did not improve between trials 3 and 4 as strongly as animals with smaller lesions. Trial 3 is the first trial the "rule" (association between cue and platform) can be known. In trial 1, there is the possibility for different rules (cue or spatial location), and trial 2 confirms the rule. While the number of subjects is too low to draw conclusions, this raises the possibility of a pPPT role in cued version of Morris water maze. Apart from this slightly worse performance on day 1 of cued paradigm, no differences in water maze performance were present between control and IBO animals in posterior groups. Neither control nor IBO posterior animals were impaired in the spatial version of the Morris water maze, consistent with expectations. Since there was no impairment in the spatial version, the slower performance on day 1 of cued task is likely not due to deficits in spatial navigation in general, but rather specific to the cued paradigm. The number of animals included in posterior IBO group with sufficient lesions should be increased to determine whether this impact on performance in cued maze was observed by chance or due to actual role of pPPT in cued Morris water maze.

In addition to using a stronger toxin to cause more extensive lesions, running a more difficult testing paradigm would lengthen the learning process so that differences were more observable. We used only one cue, consistent with other studies. It may be possible to make the test more demanding by having multiple cues within the water maze, only one of which is associated with the escape platform. This would require more robust reinforcement learning and therefore may take longer to learn, be more sensitive to deficits and/or less rescued by compensation.

Elucidating the role of cholinergic PPT cells in dorsal striatal learning may identify the contribution of PPT degeneration to cognitive deficits in Parkinson's disease. Understanding the neural basis of cognitive decline in PD can help to identify early warnings signs of the degenerative process that may present before motor symptoms.

Chapter 5

5 Conclusions

The greatest constraint of this data is the limited extent of lesions, especially in the anterior group. A more potent excitotoxin should be used in future studies, unless concentration of 0.12M IBO can be reached. Despite the relative lack of lesions, we were able to correlate cell loss with behavioral data. In line with expectations, there were no locomotor deficits in IBO animals relative to controls in either anterior or posterior groups. While no differences existed in anterior groups, both initial and habituated startle amplitudes were reduced in posterior IBO animals, confounding the correlations between lesion score and STH/LTH scores. While no correlations or differences in PPI existed for anterior groups, PPI at a 75dB prepulse and 10 or 30ms ISI was significantly correlated to lesion score, suggesting there may be a relationship between integrity of pPPT and PPI. These results are consistent with a role of posterior PPT regions in PPI. Likely due to insufficient lesions, there were no significant differences in performance in cued Morris water maze between control and aPPT-lesioned animals. Similarly, there were no deficits of pPPT-lesioned animals in cued version, although there is slight indication that more extensive lesions of pPPT can impair performance in this task. There were no differences in performance in spatial version of water maze task in either anterior or posterior groups.

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Appendices

The following are lesion scores for both sides for each animal. The score used was the lower of the two.

Appendix 1: Left and right lesion scores for anterior subjects.

	Left	Right		Left	Right
AS13	1.112	0.867	**AI13**	0.974	1.080
AS14	0.906	1.052	**AI14**	0.811	0.801
AS15	1.168	1.247	**AI15**	0.921	0.793
AS16	1.103	1.072	**AI16**	0.787	0.957
AS17	0.912	1.091	**AI17**	0.810	0.883
AS18	0.976	0.939	**AI18**	1.033	0.810
AS19	0.873	0.868	**AI19**	0.887	0.729
AS20	0.996	0.782	**AI20**	0.896	0.902
AS21	1.243	0.894	**AI21**	0.763	0.763
AS22	1.001	0.985	**AI22**	0.768	0.785
			AI23	0.891	0.906
			AI24	0.684	0.973
			AI25	0.729	0.784

Appendix 2: Left and right lesion scores for posterior groups.

	Left	Right		Left	Right
PS13	0.789	0.965	**PI13**	0.746	0.782
PS14	0.877	1.034	**PI14**	0.975	0.682
PS15	0.972	0.923	**PI15**	0.390	0.550
PS16	0.982	1.056	**PI16**	1.101	1.147
PS17	0.933	0.853	**PI17**	0.827	0.855
PS18	0.998	0.962	**PI18**	0.740	0.468
PS19	1.094	1.068	**PI19**	0.816	0.844
PS20	1.094	1.117	**PI20**	0.439	0.855
PS21	0.930	0.965	**PI21**	0.732	0.625
PS22	1.022	0.845	**PI22**	0.641	0.709

Curriculum Vitae

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