Cholinergic Effects On Ocular Flutter In Guinea Pigs Following Nerve Agent Exposure: A Review

Patrick T. Williams* and Corey J. Hilmas

Neurobehavioral Toxicology Branch / Analytical Toxicology Division
U.S. Army Medical Research Institute of Chemical Defense
3100 Ricketts Point Road
Aberdeen Proving Ground, Maryland 21010

* Corresponding Author: Tel: (410) 436-2803 | Fax: (410) 436-1960 | Email: patrick.williams23@us.army.mil


ABSTRACT
Horizontal nystagmus is a sustaining, abnormal eye movement and an early clinical sign of toxicity following nerve agent exposure. Nerve agent exposure-induced nystagmus can degenerate into ocular flutter (2 – 3 Hz) in laboratory animal experiments. Ocular flutter can be reversible which is curious given the irreversibility of acetylcholinesterase inhibition by nerve agents. Reversed ocular flutter can be followed by a return of horizontal nystagmus for a limited period. As cholinergic neurotransmission has been implicated in the initiation and control of saccadic eye movements, we review oculomotor circuits and propose a mechanism for ocular flutter involving the cholinergic input to the intermediate layer of the superior colliculus. The reversible nature of ocular flutter may be due to desensitization of nicotinic cholinergic receptor subtype populations involved in partly modulating saccade generation.

INTRODUCTION
Nerve agents are potent inhibitors of cholinesterase enzymes. Exposure to cholinesterase inhibitors can lead to a variety of clinical features indicative of an excess of the neurotransmitter acetylcholine. In addition to the most common and overt clinical signs of cholinesterase inhibition, the signs field personnel are trained to observe for initiation of ‘buddy-aid’ or the administration of first aid for nerve agent exposure, other more subtle signs of exposure can also manifest themselves. Eye movements can be one of the subtle clinical signs of organophosphorous (OP) exposure. Saccades are normal eye movements, quick phase movements that rapidly reposition the globe of the eye to orient the fovea to a visual target [Milder and Billson 1989]. They are fast and accurate eye movements characterized by low latency (150 – 250 ms), high velocity (to 600° · sec⁻¹), abrupt termination, and absence of ocular drift [Ramat et al., 2007; Milder and Billson, 1989]. Abnormalities of saccadic movements can result from, tumors [Helmchen et al., 2003; Digne 1986], viral encephalitis [Digne, 1986], lesions [Schon et al., 2001; Averbuch-Heller et al., 1996; Thurtell et al., 2007], and chemical exposures [Liang et al., 2003; De Bleecker, 1992; Pullicino, 1989]. Clinical findings related to abnormal eye movements have been reported in cases of accidental human intoxication with OP pesticides [Liang et al., 2003; De Bleecker, 1992; Pullicino, 1989]. OP pesticides like many chemical warfare agents are powerful inhibitors of acetylcholinesterase (AChE), the enzyme that hydrolyzes acetylcholine released at the neuromuscular junction, ganglia, and nicotinic/muscarinic synapses of the central nervous system.
Nystagmus is an abnormal saccadic eye movement, characterized by an involuntary slow phase drift from center with a fast phase correction [Milder and Bilson, 1989]. Nystagmus can be either unilateral or bilateral and vertical, horizontal, or torsional [Henn, 1992]. Related abnormal eye movements include ocular flutter which is defined as saccades in the horizontal direction in the absence of an intersaccadic interval [Vignaendra, 1977]. Ocular flutter is sometimes mistakenly used interchangeably with opsoclonus, though they describe rather distinct phenomena. Both terms describe saccadic oscillations in the absence of an intersaccadic interval. Ocular flutter is used to describe horizontal oscillations, while opsoclonus is reserved for combined horizontal, vertical, and torsional eye movements [Helmchen et al., 2003].

We have observed in experiments a nerve agent-induced bilateral, horizontal nystagmus, which rapidly degenerates into ocular flutter, as a subtle yet significant sign in guinea pigs undergoing cholinergic crisis, resulting from exposure to 1LD50 and 2 LD50 of VX and Soman (GD), prototypical OP-type chemical warfare agents. The intent of this review is to discuss oculomotor pathways in the brain and identify elements in these pathways susceptible to cholinergic perturbation. In addition, we speculate on the possible underlying cholinergic mechanisms involved in these abnormal eye movements.

DISCUSSION

Brainstem Saccadic Generator

The motor circuits for saccades lie in the brainstem. Horizontal saccades are generated in the paramedian pontine reticular formation (PPRF) [Luschei, 1972], whereas vertical saccades are generated in the mesencephalic reticular formation (MRF) [Büttner, 1977]. Because nystagmus and ocular flutter are disorders of saccades, this discussion will focus on the neuroanatomy and circuits related to horizontal saccades. The brainstem saccadic generator receives premotor input from a variety of structures, most importantly the superior colliculus (SC) and omnipause neurons (OPNs) from the dorsal raphe nucleus (DRN) [Zee and Robinson, 1979; Liang et al., 2003]. In turn, the saccadic generator projects to cranial nerve nuclei innervating extra-ocular muscles. Cranial nerves (CN) of the abducens nucleus (CN VI) innervate the lateral recti muscles of the eye to facilitate abduction of the eyeball. Adduction of the eyeball towards the midline is accomplished by the oculomotor nucleus (CN III).

Coordinated Horizontal Eye Movement

Excitatory burst neurons (EBNs) within the PPRF make excitatory synaptic connections with motor neurons (MNs) and interneurons (INs) of the ipsilateral abducens nucleus [Highstein, 1978; Steiger, 1979] (Figure 1). EBN activation of the ipsilateral abducens nuclei will result in abduction of the lateral rectus muscle, innervated by alpha motor neurons (MNs) of the abducens nuclei. Additionally, EBNs drive ipsilateral inhibitory burst neurons (IBNs) of the PPRF; these IBNs project monosynthetically and make inhibitory synapses with the contralateral abducens nucleus [Hikosaka, 1978; Hikosaka, 1980], preventing simultaneous activation of left and right lateral recti, resulting in divergent eye movement. Coordinated eye movement occurs with input from the medial longitudinal fasciculus (MLF) [Frohman, 2008]. Activation of ipsilateral INs in the abducens nucleus by the EBN results in activation of motor neurons in the contralateral oculomotor nucleus. Axons from these INs decussate and ascend in the MLF to synapse onto alpha MNs of the oculomotor nucleus (OMN). These MNs innervate the medial rectus of the eye such that contraction of the lateral rectus in one eye is tightly coordinated with contraction of the medial rectus in the other eye.

Control of the Saccadic Generator

SC is a complex, laminar structure that receives and integrates inputs from visual and cortical brain regions and itself makes a number of reciprocal and projective connections [Wurtz,
The parabigeminal nucleus (PBN); in turn, PBN sends cholinergic projections as well as direct visual input from the retina and Hartwich horizontal saccades, the cerebral cortex controls the saccadic generator through the SC. Integration of Visual and Motor Information by the Superostral portion of SC. These fixation neurons (FN) are active during fixation and drive the OPN to brake the premotor activity. This anatomical arrangement suggests that SC is arranged into fixation zones and saccade zones. Alternative hypotheses of SC functional organization suggest that SC computes an “error signal” in eye position by comparing the location of the fovea to that of the visual target. SC eye movement commands comprise a distance signal, encoding the difference in position between the retina and the visual target. The theories of computational function of SC are not intended to be settled here; however, based upon anatomical and physiological evidence of SGI projections to distinct premotor saccadic regions and the established modulatory effect of ACh on SC neurons, we propose the model in figure 2 to explain the observed abnormal saccadic eye movements due to cholinergic excess.

Projection neurons in the intermediate grey layer of SC (SGI) cross the midline at the dorsal segmental decussation and send glutamatergic projections to several nuclei including MRf, the contralateral LLBN, and spinal cord. Therefore, EBNs receive excitatory drive input indirectly from the SC via long lead burst neurons (LLBN) in PPRF. EBNs are tonically inhibited by glycinergic inputs from OPNs located in the DRN. OPNs comprise the brake system on the saccade generator. OPNs fire tonically during fixation with very high frequency, approaching 100 Hz, before a saccade. The suspension of OPN activity releases EBN from inhibitory input, thus allowing the activation of ABN motor neurons. The effect of OPN activity on the generation of saccades has been shown by in vivo recording and by in vitro labeling experiments, and OPNs make various complex and important contributions to the accuracy and speed of saccadic movements.

Integration of Visual and Motor Information by the Superior Colliculus

While the pontine burst circuits provide motor signals to drive the extraocular muscles for horizontal saccades, the cerebral cortex controls the saccadic generator through the SC and OPNs of DRN. SC as a whole is retinotopically organized. The three anatomical regions of the SC are the superficial, intermediate, and deep layers. The superficial layers receive visual stimuli, while the intermediate and deep layers of SC are primarily involved in oculomotor functions. Superficial layers of SC (sSC), in fact, encompass three cellular zone layers of the SC: a zonal layer (SZ); a grey layer, termed the stratum griseum superficiale (SGS); and the optic layer (SO). Together sSC receives visual input from the retinal and visual cortex. The laminae of the intermediate layer of SC, deep to SO, contain output projection neurons generally referred to as the stratum griseum intermediale (SGI). Projection neurons reside in SGI, constitute the majority of SC innervation to the rest of the brain, including the premotor saccade generator and the OPNs of DRN, and are non-GABAergic in nature. A third deep layer is found below SGI. The importance of SC in both vertical and horizontal saccades is evident by the projections that SC sends to all of the saccade-related brain regions. SC sends to all of the saccade-related brain regions (reviewed in May, 2006).

SC also has a rostral-caudal functional organization in which the deep layers in each pole seemingly send projections to different cell populations in pontine nuclei. The rostral pole sends a disproportionate amount of fibers to the OPN, compared to the caudal pole. These fixation neurons (FN) are active during fixation and drive the OPN to brake the premotor activity. This anatomical arrangement suggests that SC is arranged into fixation zones and saccade zones. Alternative hypotheses of SC functional organization suggest that SC computes an “error signal” in eye position by comparing the location of the fovea to that of the visual target.
Lund, 1982; Sefton and Martin, 1984; Mufson et al., 1986; Hall et al., 1989; Baizer et al., 1991; Feig and Harting, 1992; Jiang et al., 1996; Endo et al., 2005]. The importance of PBN will be described below.

PBN Control of Saccades Through a Direct Visuomotor Pathway

Two major cholinergic projections to the SC, the parabigeminal nucleus (PBN) and the pedunculopontine tegmental nucleus (PPTN), can modulate and gate excitatory projections from SC. While the functional role of PBN has not been studied, this region is believed to respond to visual stimuli presented in the receptive field. PBN, the principal source of cholinergic input to sSC [Hall et al., 1989; Mufson et al., 1986] integrates visual information to sSC directly, but it is not understood how that visual information is transmitted to eye movement. The connection between sSC and SGI, the main outflow from SC, was thought not to exist [Edwards, 1980]. Support for the hypothesized vertical connection between the two layers [Robinson, 1972; Sprague, 1975] to form a direct visuomotor pathway does exist from anatomical [Mooney et al., 1988; Rhoades et al., 1989; Behan and Appel, 1992; Hall and Lee, 1993; Lee and Hall, 1995] and electrophysiological studies [Ishii et al., 1998; Endo et al., 2005; Lee et al., 2007]. Cholinergic fibers from PBN may project to GABAergic neurons in sSC [Binns and Salt, 1994; Lee et al., 2001; Endo et al., 2005]. Several subtypes, including type IA fast-desensitizing α7, of nicotinic acetylcholine receptors (nAChRs) are located presynaptically to modulate the release of GABA from GABAergic interneurons of sSC to dendrites of SGI projection neurons [Endo et al., 2005]. Therefore, cholinergic input from PBN may serve to inhibit projection neurons from SGI. Whether these projection neurons are FNs of the rostral SGI projecting to the contralateral OPN is unclear at this point. It is quite possible that cholinergic fibers from PBN facilitate inhibition of excitatory projection FNs to the OPN; therefore, the OPN is not activated to inhibit the saccade generator in PPRF.

Cholinergic Effects on Saccade Generation from the Pedunculopontine Tegmental Nucleus (PPTN)

There is an abundance of cholinergic neurotransmission projecting into and within the brain regions controlling saccadic eye movements. Perturbation of AChE activity at these sites by nerve agent could disturb the precise control between saccade generation and visual fixation, leading to the observed eye movement abnormality.

PPTN, part of the reticular activating system of the brainstem [Kobayashi et al., 2002], contains an ensemble of cholinergic and glutamatergic neurons [Hallanger and Wainer, 1988] surrounding the superior cerebellar peduncle. The evidence for PPTN influencing saccadic eye movement comes from several lines. Frontal eye field neurons and substantia nigra pars reticulata, regions known to control eye movement and saccades, project to the PPTN [Gerfen et al., 1982; Hikosaka and Wurtz, 1983; Bruce and Goldberg, 1985; Granata and Kitai, 1991; Shouse and Siegel, 1992; Matsumura et al., 2000; Kobayashi et al., 2001]. SC receives a strong cholinergic input from PPTN in a variety of mammalian species [Graybiel, 1978; Beninato and Spencer, 1986; Hall et al., 1989; Henderson and Sherriff, 1991; Ma et al., 1991; Schnurr et al., 1992; Jeon et al., 1993]. In particular, SGI receives the cholinergic input from PPTN [Billet et al., 1999]. Microinjection of nicotine into the SC of monkeys shortens reaction times of visually guided saccades [Aizawa et al., 1999], demonstrating the importance of cholinergic projections to the SC on saccade generation. PPTN is also thought to integrate saccade related signals from other regions to the SC, which in turn, relays them to PPRF for saccade generation [Kobayashi et al., 2002].

The abnormal eye movements were surprisingly reversible during the period of observation. This is most likely due to desensitization of the nicotinic receptor subtypes found on superior colliculus neurons. The α7 subtype, activated by ACh or choline [Alkondon et al., 1997] located presynaptically on GABAergic interneurons of sSC [Endo et al., 2005] is rapidly desensitizing. Desensitized α7 receptors do not continue to activate in the presence of ACh (or choline) because of the change to a non-conducting state. Therefore, despite the presence of
ACh, secondary to AChE inhibition by nerve agent, the nicotinic receptors no longer transmit the signal downstream. During desensitization, OPNs should gate the saccade generator once more, and LLBNs will not be activated by SC projection neurons to drive the EBN saccade generator. Since ocular flutter lasts 10-20 minutes, other nAChR subtypes besides fast-desensitizing α7 must be involved. GABAergic neurons in SC were also shown to contain α3β2 and α6β2 nAChR subtypes [Endo et al., 2005].

Soman exposure resulted in roughly twice as many cases of horizontal nystagmus/ocular flutter than did VX exposure. This is most likely related to the difference between VX and soman in their ability to form a covalent bond to AChE. This aging process occurs immediately in the case of soman, compared to hours for VX. The oxime, 2-PAM, is much more effective in releasing AChE from nerve agent in the case of VX. Thus, soman is more toxic than VX even in the presence of 2-PAM. It is not surprising therefore that soman increased the likelihood of horizontal saccades and ocular flutter developing.

We propose that ocular flutter in nerve agent-exposed guinea pigs and other clinical findings of abnormal saccadic eye movements following exposure to OP-type pesticides are due to hyperexcitability and over stimulation of the saccadic generator circuits secondary to AChE inhibition. The combination of cholinergic potentiation within SGI, to directly drive LLBNs of the saccadic generator, and loss of OPN inhibitory drive to EBN is sufficient to cause ocular flutter in a percentage of exposed animals.
FIGURES

Figure 1. Generation of horizontal eye movements.

EBN within the PPRF send excitatory projections to both alpha motor neurons and interneurons in the ipsilateral ABN. ABN motor neurons cause abduction of the ipsilateral eye by contraction of the lateral rectus and interneurons cause adduction of the contralateral eye by excitatory projections to motor neurons in the contralateral OMN that contract the medial rectus muscle. EBN also make excitatory synapses on IBN within the PPRF, which make inhibitory connections on motor neurons in the contralateral ABN, thereby preventing divergence movements. EBN are tonically inhibited by projections from the OPN, which prevent eye movement during fixation. EBN are themselves driven by LLBN, also in the PPRF. LLBN will be the basis of functional connection between this premotor saccadic generator and SC, a saccade command generating region. (Adapted from Goldberg, 2000).
Figure 2. ACh modulation of SC output.

Two main cholinergic inputs to SC are from PBN and PPTN. PBN sends cholinergic inputs to GABAergic interneurons in superficial SC. FN projection neurons are glutamatergic and make excitatory synapses as shown.

LIST OF ABBREVIATIONS

Note: throughout the text, abbreviations not specifically referring to brain or anatomical regions will appear in italics.

2-PAM 2 praladoxime chloride; ABN abducens nucleus; ACh acetylcholine; AChE acetylcholinesterase; CN cranial nerve; DRN dorsal raphe nucleus; EBN excitatory burst neuron; FN fixation neuron; GD soman; IBN inhibitory burst neuron; IN interneuron; LLBN long lead burst neuron; MLF medial longitudinal fasciculus; MN motor neuron; MRF mesencephalic reticular formation; nAChR nicotinic acetylcholine receptor; OP organophosphorous; OPN omnipause neuron; OMN oculomotor nucleus; PBN parabigeminal nucleus; PPRF parapontine reticular formation; PPTN pedunculopontine tegmental nucleus; SC superior colliculus; sSC superficial
superior colliculus; **SGI** stratum griseum intermediale; **SGS** stratum griseum superficiale; **SO** optic layer; **SZ** zonal layer

REFERENCES


K.E. Binns and T.E. Salt (1994) Excitatory amino acid receptors participate in synaptic transmission of visual responses inn the superficial layers of the cat superior colliculus, Eur J Neurosci. 6, 161-169.


M.N. Schouse and J.M. Siegel (1992) Pontine regulation of REM sleep components in cats: integrity of the pedunculopontine tegmentum (PPT) is important for phasic events but unnecessary for atonia during REM sleep. Brain Res. 571, 50-63.


