Brief Report

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Gulf War Syndrome: A role for organophosphate induced plasticity of locus coeruleus neurons

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Gulf War syndrome is a chronic multi-symptom illness that inflicted about a quarter of the deployed veterans of the 1991 Gulf War. Exposure to prolonged low-level organophosphate insecticides and other toxic chemicals is now thought to be responsible. Chlorpyrifos was one commonly used insecticide. The metabolite of chlorpyrifos, chlorpyrifos oxon is a potent irreversible inhibitor of acetylcholinesterase, much like the nerve agent Sarin. To date, the target brain region(s) most susceptible to the neuroactive effects of chlorpyrifos oxon have yet to be identified. To address this we tested ability of chlorpyrifos oxon to influence neuronal excitability and induce lasting changes in the locus coeruleus, a brain region implicated in anxiety, substance use, attention and emotional response to stress. Here we used an ex vivo rodent model to identify a dramatic effect of chlorpyrifos oxon on locus coeruleus noradrenergic neuronal activity. Prolonged exposure to chlorpyrifos oxon caused acute inhibition and a lasting rebound excitatory state expressed days after withdrawal. Our findings indicate that the locus coeruleus is a brain region vulnerable to chlorpyrifos oxon-induced neuroplastic changes possibly leading to the psychological symptoms and motor pathologies inflicting veterans of the Gulf War.

Gulf War deployment has been associated with an increased prevalence of psychological symptoms, such as anxiety and depression, substance use, mental disorders and a lower quality of life beginning during the war and persisting a decade later. In 2008, a US Government panel concluded and validated that "Gulf War syndrome" (GWS) afflicted about 25% of the 700,000 deployed US veterans[1]. Epidemiological studies indicate that repetitive low-level exposure to organophosphate chemicals, including the insecticide, chlorpyrifos (CP) was one probable causative factor in GWS[2].

CP was frequently used as an insecticide applied to bedding and clothing during the Gulf War. Since the Gulf War the use of CP has been banned for household use in the US, but use and exposure remains high in agricultural communities and developing countries. Epidemiological data from agricultural communities that use CP regularly also report GWS-like symptoms. Lasting developmental delays in children exposed to CP have been confirmed along with a 50-75% increase in the prevalence of Attention Deficit Hyperactivity Disorder (ADHD) per 10 fold increase in urinary metabolites of CP.[1,3]

CP is metabolized and excreted in urine as the toxic metabolite chlorpyrifos oxon (CPO). CPO is several fold more potent than CP as a neurotoxin due to its irreversible inhibition of acetylcholinesterase (AChE) activity, a similar mechanism to the neurotoxin, Sarin[4,5]. Given the lack of a direct connection between CPO and changes in neuronal function we developed an ex vivo rodent brain slice preparation to assess the effects of acute and prolonged exposure effects of CPO on the excitability of noradrenergic neurons within the LC. The LC provides the sole source of noradrenaline in the brain and has a well-established role in mediating arousal, attention, anxiety and stress response[1].

RESULTS

The results show that CPO (50 uM) application to cultured brain slices containing the LC significantly (n=12; p<0.01) reduced the firing rate of putative noradrenergic neurons (**fig. 1a**) recorded using extracellular loose seal patch recording at 33°C. The CPO-induced effect fully washed out after 30 minutes indicating it was not directly toxic to the brain slice.

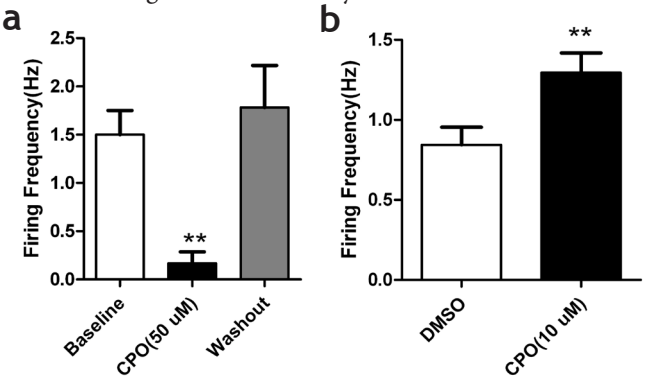

Figure 1: **(a)** Acute application of CPO reduces spontaneous action potential activity in the LC noradrenergic neurons. Loose seal extracellular patch-clamp recordings were obtained from brain slices 250 µm thick. CPO (50 µm) applied for 10 minutes substantially reduced firing rate (n=12, **p < 0.01) before being washed out for 30 minutes. **(b)** Three day treatment with CPO (10 µm) or vehicle (DMSO) elicited a rebound increased basal LC neuronal activity when CPO was removed from the slice (n=10-12,**P < 0.01). http://www.neurocloud.org/nature-precedings/cao/

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In a second experiment (**fig 1b**) we tested the effects of prolonged (3 days) exposure to lower doses of CPO (10 μM). After a 3 days of CPO and a 90-180 minutes washout period we observed a rebound increase in the activity of putative noradrenaline neurons within the LC indicating a lasting plasticity resulting from prolonged exposure to CPO. Our results indicate that as a model system, the LC slice cultures are ideal for addressing whether acute and prolonged CPO exposure can induce homeostatic changes in LC excitability ex vivo and further identify the molecular mechanisms responsible for the plasticity.

DISCUSSION

We identified a possible physiological mechanism underlying a subset of the psychological symptoms associated with GWS. This study provides the first evidence showing that the toxic metabolite CPO of the insecticide CP, alters LC noradrenergic neural activity in a lasting manner. Deployed Gulf War soldiers were overexposed to insecticides and the GWS symptoms are reported to be higher amongst those exposed to organophosphates (OP). Government reports indicate that insecticides were applied on skin, clothing, in tents, sleeping quarters and sprayed in camp[1]. To make the problem worse oxidative conversion of CP to the more toxic CPO occurs when CP comes into contact with oxidizing decontaminating cleaning and laundry products, such as bleach. The exact mechanism for how prolonged low-level exposure leads to psychiatric symptoms in some individuals and not others remains unknown.

Recently, however, the OP metabolic pathway has been implicated as a genetic vulnerability factor for some individuals. Studies have found that Gulf War veterans who became chronically ill have lower levels of the enzyme paroxanase I (PONI), which serves to neutralize nerve agents and OP compounds like CPO[1]. Individual genetic polymorphisms in PONI may contribute to the severity of symptoms reported in those exposed to low-levels of OP compounds.
The possibility that certain individuals might be vulnerable to low-levels of OP insecticides is a concern from a human health and public policy perspective because OP pesticides are still widely used in agriculture communities and the developing world. In fact, recent reports found that environmental CP exposure produces developmental delays in children and substantially increases the prevalence of ADHD[1,3]. In light of this there is an urgent need to develop better ways to test for OP contaminants in the environment and understand their physiological effects in model systems.

In the brain slice model we found robust modulation of neural excitability in neurons within the LC. Acute application of CPO produced a rapid inhibition of spontaneous activity followed by a prolonged excitation lasting days. Although we did not assess the behavioral state of rats exposed to CPO, we have observed similar lasting changes in rat LC neural activity after acute and prolonged morphine exposure. During withdrawal from morphine there is a profound withdrawal syndrome that is correlated with a prolonged increase in the excitability of LC neurons and behavioural states of anxiety and distress[7]. Future research is needed to determine if withdrawal from prolonged CPO exposure in vivo results in the same molecular mechanism underlying withdrawal and anxiety from morphine[1].

It is likely that pathological changes in the activity of LC neurons during CPO withdrawal disrupt the balance of noradrenaline throughout the brain and especially in brain regions responsive to stress. This change in LC output may lead to increased susceptibility to stressful traumatic events. Understanding the cellular neuroadaptations in response to low-level CPO exposure in the LC neurons is important for developing treatments with the potential to reverse pathological plasticity of LC excitability and the psychological states associated with it.

METHODS

Slice Cultures and Recordings

Slice cultures were prepared using the traditional method first introduced by Stoppinni et al. (1991)[6]. Acute LC slices were trimmed as thin as possible and maintain for 3d in an incubator at 33°C. A Gibco MEM medium consisting of 30 mM HEPES, 20 mM D-glucose, 5% B27, 5.0 mM L-glutamine and 25 U/ml streptomycin/penicillin based on previous LC culture which proved sucessful[6]. Slice cultures were measured in aCSF with a DigiData 1233A and pClamp8 (axon instruments). For expanded methods and recordings please see http://www.neurocloud.org/nature-precedings/cao/

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PROGRESS AND COLLABORATIONS

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AUTHOR CONTRIBUTIONS

J.L.C. & D.C.C designed the experiment, J.L.C. prepared the cultures, and recorded and analyzed the data. J.L.C., A.L.V. & D.C.C. wrote and prepared the manuscript.

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