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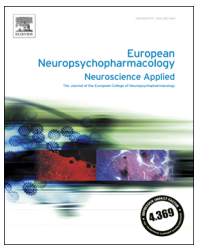

Differential effect of clomipramine on habituation and prepulse inhibition in dominant versus subordinate rats

Alvin Yang, Tahira Daya, Karen Carlton, Jin Hui Yan, Susanne Schmid*

Anatomy and Cell Biology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada

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Abstract
Many patients with depression have comorbidities associated with an impairment of sensorimotor gating, such as e.g. schizophrenia, Parkinson Disease, or Alzheimer disease. Antidepressants like clomipramine that modulate serotonergic or norepinephrinergic neurotransmission have been shown to impact sensorimotor gating, it is therefore important to study potential effects of clomipramine in order to rule out an exacerbation of sensorimotor gating impairment. Prior studies in animals and humans have been inconclusive. Since serotonin and norepinephrine levels are closely related to anxiety and stress levels and therefore to the social status of an animal, we tested the hypothesis that acute and chronic effects of clomipramine on sensorimotor gating are different in dominant versus subordinate rats, which might be responsible for conflicting results in past animal studies. We used habituation and prepulse inhibition (PPI) of the acoustic startle response as operational measures of sensorimotor gating. After establishing the dominant animal in pair-housed male rats, we injected clomipramine for two weeks and measured acute effects on baseline startle, habituation and PPI after the first injection and chronic effects at the end of the two weeks. Chronic treatment with clomipramine significantly increased habituation in subordinate rats, but had no effect on habituation in dominant animals. Furthermore, PPI was slightly enhanced in subordinate rats upon chronic treatment while no changes occurred in dominant animals. We conclude that the social status of an animal, and therefore the basic anxiety/stress level determines whether or not clomipramine has a beneficial effect on sensorimotor gating and discuss possible underlying mechanisms.

*correspondence to: Anatomy and Cell Biology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, N6A 5C1. Tel.: +1 519 661 2111x82668; fax: +1 519 661 3936.
E-mail address: susanne.schmid@schulich.uwo.ca (S. Schmid).

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1. Introduction

Clomipramine is widely prescribed to treat depression and anxiety disorders (Dell'Osso et al., 2006). It is a competitive antagonist that binds to monoamine transporters, so that binding of the endogenous monoamine neurotransmitter is inhibited (Apparsundaram et al., 2008). The resulting increase of monoamines at synaptic sites may cause an initial increase in anxiety levels (Browning et al., 2007; Grillon et al., 2007). As chronic treatment persists, adaptive changes seem to occur and therapeutic anxiolytic effects can be seen (Bosker et al., 2010). While many monoaminergic re-uptake inhibitors have a limited selectivity and affect serotonergic, norepinephrinergic and dopaminergic systems, clomipramine has a high potency as a serotonin and norepinephrine re-uptake inhibitor, but very limited effects on the dopaminergic system. This is important, as dopaminergic modulators are commonly known to impair sensorimotor gating. Sensorimotor gating disruptions are observed e.g. in schizophrenia, autism spectrum disorders, and many neurodegenerative diseases, and they have been shown to correlate with psychotic symptoms and/or cognitive impairments. It is therefore specifically critical that sensory gating deficiencies are not exacerbated when clomipramine or other monoamine re-uptake inhibitors are applied (Swerdlow et al., 2006b). Reports on the effects of acute or chronic monoamine re-uptake inhibitors on sensorimotor gating in animals and humans have been conflicting in the past (for details please see Section 4), probably due to differences in the specificity of re-uptake inhibitors, and their affinity for different monoaminergic systems. Another confounder might be the differences in basic anxiety or stress level among patients and human volunteers or between individual animals used in the studies. We here address the latter by exploring the effect of clomipramine in dominant versus subordinate rats, thereby taking differences in baseline anxiety and stress levels into account.

Social ranking in male rats and various other animals correlates with anxiety and stress levels: rats demonstrating lower anxiety related behaviour are considered to be more active and aggressive in social behaviour (Henniger et al., 2000) and they become dominant, as this behaviour is an important part of survival and resource allocation in social hierarchies (Davis et al., 2009). Interestingly, aggressive behaviour and serotonin activity are closely related (Jansen et al., 2011; Passamonti et al., 2012), as are social status and serotonergic activity (for review see Chiao, 2010; Whitaker et al., 2010; Morrison and Cooper, 2011; Morrison *et al.*, 2011; Issa et al., 2012). Increased stress levels also induce norepinephrine release (Hajos-Korcsok et al., 2003). We therefore hypothesise that the effect of monoamine re-uptake inhibitors might be influenced by the social status of an individual rat.

In order to address this hypothesis, we tested the acute and chronic effects of clomipramine on the acoustic startle response, habituation, and PPI in dominant versus subordinate rats. Modulations of the acoustic startle response is the most common method for studying sensorimotor gating in humans and animals, whereas the baseline startle response amplitude is an indicator of fear and anxiety levels.

2. Experimental procedures

2.1. Animals

A total of 54 Male Wistar Rats (Charles Rivers, Canada) of approximately six weeks, weighing 250–320 g at the start of the experiments were used in these studies. They were housed in cages of two animals under continuous 12 h light–dark cycles, with water *ad libitum*. The entire experiment was run three times. For the second and third run, sexually experienced rats were used and they were food restricted (20 g of standard rat chow per cage) for one week prior to the startle experiments in order to increase aggressive behaviour. All rats were weighed every two days to calculate appropriate dosages of drugs. The experiments were done in accordance with the ethical guidelines for the care and use of laboratory animals for experiments by the Canadian Council for Animal Research, and were approved by the local animal care committee (University of Western Ontario).

2.2. Study design

The entire study was run three times each with 8 pairs of rats (dominant and subordinate). An additional group of 6 rats that were injected with saline through the entire study was run concurrently with the second batch as a control group. For data analysis, we used a within subject design, comparing initial startle data with data after acute clomipramine treatment, chronic treatment, and after 2 weeks of recovery for each animal. All three experiments with drug injections showed the same results, data for each group was therefore merged and averaged across all three runs.

2.3. Determination of social status

Animals were housed in pairs and social behaviour was monitored twice daily by two independent observer, once per light and per dark cycle, for one week. Aggressive behaviour (fighting, threatening) as well as resting positions in the cages were noted and used to determine the dominant and subordinate animals (Henniger et al., 2000). Fighting was monitored amongst pairs, as the winner established his dominance through always gaining a superior position on the subordinate rat. The latter often lied on its back or submitted its head while the dominant one was stepping on top of it. Resting/sleeping locations were also observed, as dominant rats tended to rest inside a tube placed in the cage, and would fight the subordinate rat in order to establish its position. Alternatively, the dominant rat would lie on top of the subordinate. After each observation, rats within one cage were ranked dominant or subordinate or as being inconclusive. After one week of observations, dominance rankings were tallied. Animals were considered being dominant when at least 75% of observations were consistent with dominant behaviours. Data of animals in a total of three cages was omitted because no clear distinction could be determined between the dominant and subordinate rat. In the last run of 16 rats, observation of social status was repeated immediately after chronic treatment in order to see whether animals changed social ranks through the treatment.

2.4. Injections

All rats were injected subcutaneously with saline (0.9% sodium chloride) once every 12 h, for three days. Acoustic startle responses were then measured for the control treatment. Testing was repeated the next day after one subcutaneous injection of