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Short communications

Involvement of the caudal granular insular cortex in alcohol self-administration in rats

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HIGHLIGHTS

- CGIC inactivation reduced operant responding for alcohol under a fixed ratio-3 schedule.
- CGIC inactivation increased the amount of alcohol left unconsumed by the rats.
- Altogether, CGIC inactivation reduced intake of 12% alcohol in rats.

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ABSTRACT

Animal models of substance abuse have established a role for the caudal granular insular cortex (CGIC) in drug taking behaviour for several addictive substances, yet nothing has thus far been reported for alcohol. The current research was undertaken to examine the involvement of the CGIC in a rat model of alcohol self-administration. We investigated the inactivating effects of local infusions of a γ-aminobutyric acid agonist mixture (baclofen/muscimol) into the CGIC on alcohol self-administration under a fixed ratio-3 (FR-3). This inactivation of the CGIC decreased operant responding for alcohol along with a corresponding decrease in oral alcohol intake. Our results demonstrate the involvement of the CGIC in alcohol taking behaviour and suggest future studies examine the differential involvement of the various subregions of the insular cortex in various aspects of alcohol consumption.

1. Introduction

The insular cortex has been a region of great interest in addiction research since Naqvi et al. observed that stroke-induced damage to this region disrupts addiction to cigarette smoking [1]. The insular cortex, or insula, is responsible for interoception or the physiological condition of the body [2]. Understandably, insular activity has been observed in neuroimaging studies of anxiety, cognition, conscious urges, anxiety, pain, cognition, mood and substance abuse while animal models have also demonstrated a role for the insular cortex in behavioural aspects of multiple addictive substances (see [3] for review).

The granular, dysgranular, and agranular corticies are the three structurally and functionally distinct subregions that comprise the insula [4]. The caudal granular insular cortex (CGIC) integrates nociceptive [5] and unimodal viscerosensory [6] afferents from the thalamus along with those from the somatosensory cortex. It also primarily sends efferent projections back to those areas as well as to the caudate-putamen [7]. Through the dysgranular insular subregion, information initially integrated in the CGIC is relayed and further integrated before reaching the rostral agranular insular cortex (RAIC) [8]. Importantly, the granular insular cortex is the only

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insular subregion lacking projections to nuclei within the amygdala [8].

Recent findings by Seif et al. have established a role for glutamatergic neurons, projecting from the rostral agranular insular cortex (RAIC) to the nucleus accumbens, in quinine or electric footshock-based aversion-resistant alcohol intake but not for alcohol intake in the absence of such deterrents [9]. To our knowledge, no studies have yet to examine a role for the CGIC in alcohol self-administration. Thus, the current study examined whether the CGIC was involved in alcohol self-administration behavior, as we have previously observed for nicotine. The same intracranial manipulations used previously were used in the current study: bilateral infusions of γ-aminobutyric acid (GABA) receptor agonists, baclofen and muscimol.

2. Methods and results

Naive male Long-Evans rats (Charles River, Lachine, QC) weighing 300–325 g at the start of experiments were maintained on ~20 g of rat chow daily and ad libitum water while in their home cages. Animals were single-housed in a temperature-controlled room on a 12 h reverse light cycle with all behavioral testing occurring during the dark phase.

Alcohol solution for oral self-administration was prepared by diluting 95% ethanol in tap water. A mixture of baclofen and muscimol (0.3 and 0.03 nmol/side, respectively; Sigma–Aldrich) was dissolved in saline for intracranial infusions.

Procedures were similar to those previously reported [10]. Briefly, animals were initially placed in drinking cages for 30 min/day and given access to two Richter tubes containing either water or alcohol solution in increasing concentrations: 3% (w/v) for 5 days, 6% (w/v) for another 5 days, and finally 12% (w/v) for 10 days.

Animals then continued onto alcohol self-administration with the 12% (w/v) solution for 1 h daily. All operant procedures were conducted in experimental chambers with two retractable levers located on a chamber wall (Coulbourn Instruments, Allentown, PA). The start of each daily session was indicated by the extension of these two levers and the concurrent illumination of a house light on the opposing chamber wall. Animals were required to press one of the two levers, the active lever, in order to receive 0.19 ml delivered by an infusion pump (Med Associates, Model PHM-104) to a receptacle positioned between the two levers. Responding on the opposite lever, the inactive lever, was recorded but had no consequences. A cue light was present above the active lever and would illuminate for the duration of the 5 s timeout period following each reinforcement during which time the house light was turned off and responding on either lever was recorded but had no consequences. At the end of each session, the amount of alcohol remaining in the receptacle (wasted alcohol), was measured using a needle syringe and recorded. Alcohol intake (g/kg) by each rat for the session was calculated using the recorded values for the number of reinforcements earned, wasted alcohol and body weight measured before the start of the session. Rats underwent 5 sessions under a fixed ratio-1 (FR-1) schedule of reinforcement, followed by 5 sessions under an FR-2 schedule and then a minimum of 10 sessions under an FR-3 schedule (ie. three active lever presses required for each reinforcement) before receiving surgical cannulation of the CGIC. Operant responding, reinforcements and alcohol intake during training is shown in Fig. 1.

Following the acquisition of self-administration, rats underwent surgical implantation of bilateral microcannulae guide into the CGIC as follows. Rats were anaesthetised in an isoflurane (5%) induction chamber before being positioned in a stereotaxic apparatus (Kopf, Model 900) after which anaesthesia was maintained

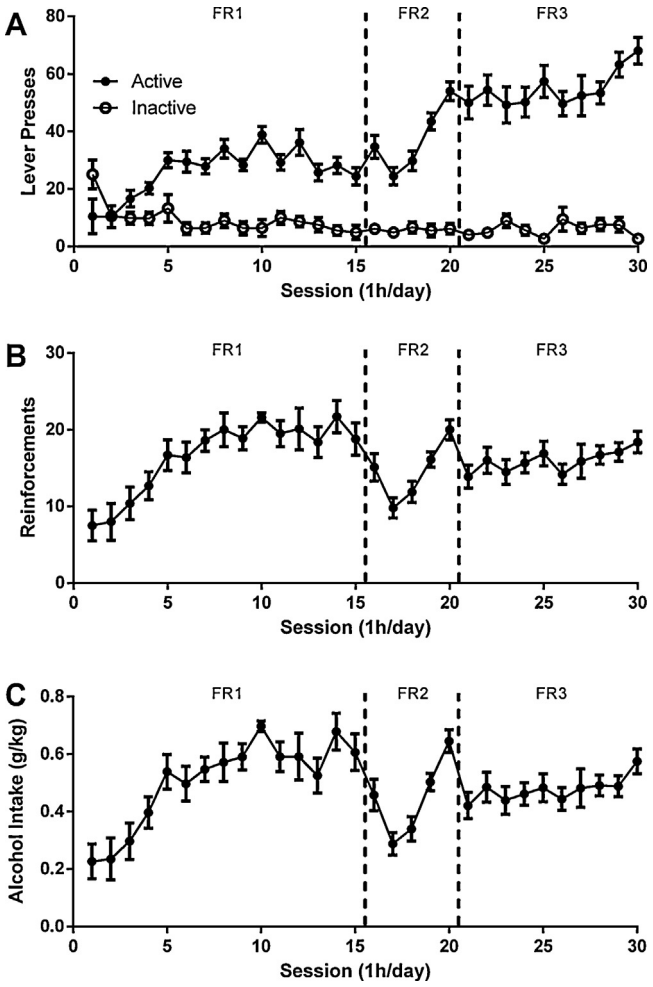

Fig. 1. Acquisition of alcohol self-administration behaviour. Active and inactive lever responding (A), reinforcements (B), and alcohol intake (C) during self-administration training at fixed ratio (FR) 1–3. Self-administration sessions under each FR are separated by dashed lines. Data are expressed as means (± SEM) of the number of lever presses, reinforcements earned or alcohol intake (g/kg).

using isoflurane (1–2%) delivered via nose cone. The rats' heads were shaved and local anesthetic (0.1 ml bupivicaine, 0.125%) was injected at the incision site before betadine was applied to clean the area. An incision was made along the midline and the skull exposed. Location of bregma and lambda were determined and the skull was levelled. Sites of interest were determined relative to bregma as follows, CGIC: anteroposterior −0.4 mm, lateral ± 4.8 mm. Small holes were drilled at the respective sites for each rat. At a 10° angle divergent from the vertical, guide microcannulae (22G; Plastics One, Roanoke, VA) were lowered 5.0 mm relative to the dorsoventral coordinate taken from the cranial surface at the site of interest. Guide cannulae were then fixed to the skull with screws and dental cement (Jet Set 4; Lang Dental, Wheeling, IL) and sealed with a stainless-steel occluder (Plastics One). Postsurgical analgesia was achieved using buprenorphine (.01 mg/kg, subcutaneous). Rats were given a minimum of 7 days recovery time in their home cages.

Following intracranial cannulation, rats were again given daily sessions of alcohol self-administration for a minimum of 5 days, until behaviour stabilized (less than 20% variation in active lever presses and alcohol intake). Once stable, rats were given either GABA agonist or vehicle infusions into the bilateral CGIC, as described, before undergoing their usual alcohol self-administration session. Following this first testing, rats underwent