

# Carbon dioxide-induced anxiety and the successive negative contrast effect in rats

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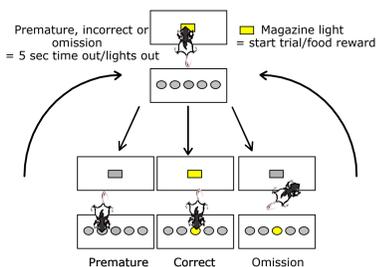
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## Background

- Evaluation of behavioural changes in response to reward gain or loss has been hypothesised to reflect underlying affective bias in animals.
- Previous studies have shown that successive negative contrast (SNC) is seen in animals where reward value is reduced relative to the expected outcome [1].
- We have demonstrated a significant effect on correct latency, collection latency and premature responding to reward loss using an operant SNC task [2].
- In this study, we investigate the effect of inducing a negative affective state in rodents using CO<sub>2</sub> inhalation.
- CO<sub>2</sub> inhalation in psychiatric research has been used as both a diagnostic tool and a way of exploring the mechanisms involved in generalised anxiety disorder and the production of panic attack.
- CO<sub>2</sub> inhalation in animals suggests that this model activates brain areas associated with anxiety circuitry [3] and we have shown an anxiogenic effect of CO<sub>2</sub> concentrations between 2 and 8% in open field activity.
- This study aims to investigate the dose-response relationship between CO<sub>2</sub> concentration and performance in an instrumental SNC task.

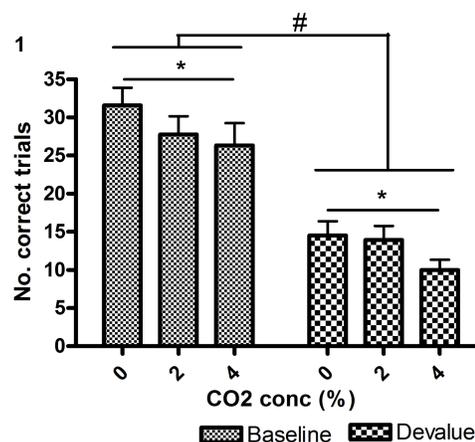
## Method

- Male Lister-hooded rats were trained and tested using standard Med Associates 5-hole boxes and KLimbic software (Conclusive solutions Ltd).
- Rats were housed in groups of four on 12 h reverse lighting. Rats were food restricted to maintain them at 85% of their free-feeding weights.
- Animals were trained to respond to a visual cue with nose-poke responses under a progressive ratio schedule to receive a four pellet reward.
- At the end of training, a series of devalue sessions were introduced where animals received only a single pellet outcome for each correct response.
- Animals were then run in the task under baseline and devalue conditions under normal air or 2 or 4% CO<sub>2</sub> using a fully randomised within-subject study design. A further study was then carried out using air vs. 6% CO<sub>2</sub>.

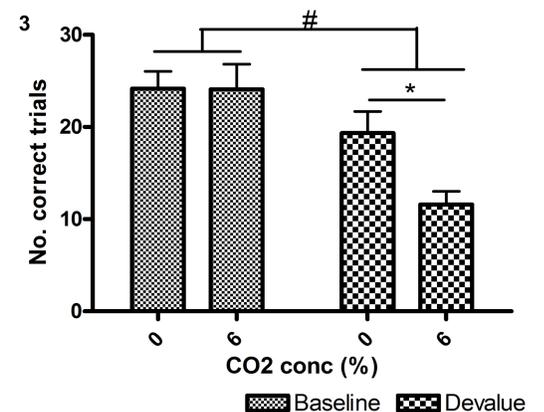


**Figure 1:** Diagrammatic representation of the instrumental successive negative contrast (iSNC) task. For the iSNC protocol a modified forced choice serial reaction time task (FCSRTT) was used. Animals initiate a trial by nose-poking in the food magazine. Following a pre-set inter-trial interval (ITI) of 20 sec, animals are presented with a visual stimulus (5 sec) in the central nose-poke aperture. Responses are recorded as correct, omission or premature. Perseverative nosepokes, correct latency (time taken to respond to the stimulus/ motivation to respond) and collection latency (motivation to collect reward) are also recorded. Animals had to respond under a progressive ratio schedule. Break point was recorded as the number of correct responses before the animal failed to make a response for 5 minutes.

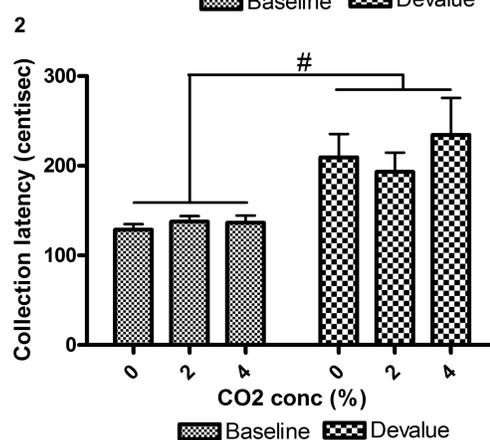
**Figure 1:** Effect of inhalation of 2 or 4% CO<sub>2</sub> on performance in the iSNC task. A significant main effect of SESSION ( $F_{(1, 11)} = 87.44$ ;  $P < 0.0001$ ) and TREATMENT ( $F_{(1, 11)} = 5.25$ ;  $P < 0.014$ ) was seen with both devaluing of reward and 4% CO<sub>2</sub> inhalation resulting in a reduction in correct trials.



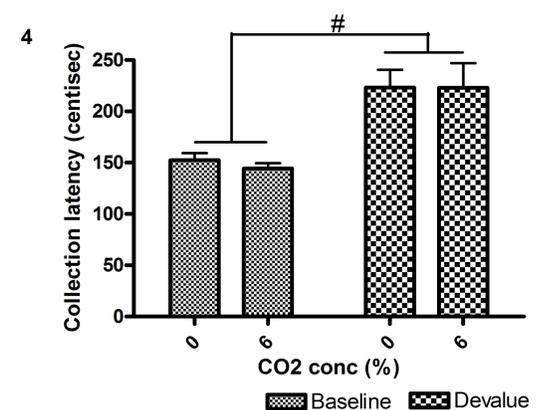
**Figure 3:** Inhalation of 6% CO<sub>2</sub> enhances the contrast effect seen when reward is reduced from 4 to 1 pellet. A significant main effect of SESSION ( $F_{(1, 11)} = 35.14$ ;  $P < 0.0001$ ) demonstrated the SNC effect. A main effect of TREATMENT ( $F_{(1, 11)} = 4.89$ ;  $P < 0.049$ ) was also seen. There was a trend towards a SESSION\*TREATMENT interaction ( $F_{(1, 11)} = 4.39$ ;  $P < 0.060$ ) where 6% CO<sub>2</sub> appears to selectively reduce correct trials under devalue conditions.



**Figure 2:** The contrast effect seen in collection latency was not affected by CO<sub>2</sub> inhalation. A significant main effect of SESSION ( $F_{(1, 11)} = 20.69$ ;  $P < 0.0001$ ) was seen but there was no main effect of TREATMENT ( $F_{(2, 122)} = 0.47$ ;  $P = 0.633$ ) or SESSION\*TREATMENT interaction ( $F_{(2, 22)} = 0.49$ ;  $P = 0.62$ ).



**Figure 4:** Inhalation of 6% CO<sub>2</sub> had no effect on the contrast effect in collection latency. A significant main effect of SESSION ( $F_{(1, 11)} = 6.09$ ;  $P = 0.031$ ) was present but there was no main effect of TREATMENT ( $F_{(1, 11)} = 1.19$ ;  $P = 0.298$ ) or SESSION\*TREATMENT interaction ( $F_{(1, 11)} = 0.833$ ;  $P = 0.38$ ).



## Discussion

- Results from inhalation of 2% or 4% CO<sub>2</sub> show that both devaluing of reward and CO<sub>2</sub> inhalation result in a reduction in correct trials. This indicates a reduced motivation to perform the task.
- A similar pattern was seen with 6% CO<sub>2</sub> inhalation vs. air where there was also a trend towards a SESSION\*TREATMENT interaction with 6% CO<sub>2</sub> appearing to selectively reduce correct trials under devalue conditions.
- A significant main effect of session was observed demonstrating the SNC effect for all other recorded variables but these were unaffected by CO<sub>2</sub> induced anxiety.
- Motivation to perform the task but not to collect the reward appears to be affected by induction of negative affective state.

## CONCLUSION

**Induction of negative affective state using CO<sub>2</sub> affects performance in the instrumental SNC task. A reduction in correct trials suggests that induction of anxiety affects motivation to perform the task. Collection latency was unaltered indicating that motivation to collect the reward was not affected by anxiety and that this component of the SNC effect is not sensitive to affective state.**

- [1] Flaherty CF. Incentive Relativity. New York: Cambridge University Press; 1996  
 [2] Mitchell et al, 2010. Manipulation of environmental conditions alters the magnitude of the instrumental successive negative contrast effect in rats. BAP Summer Meeting 2010. Supplement to Journal of Psychopharmacology, vol 24. Supplement 3, A77.  
 [3] Lowry, C. A., P. L. Johnson, et al. (2005). "Modulation of anxiety circuits by serotonergic systems." Stress 8(4): 233-46

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