A high-throughput mouse stop signal task (SST)

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The stop signal task is a widely used test in humans to measure behavioral inhibition and, more specifically, action cancellation which can be affected in disorders such as obsessive compulsive disorder. An equivalent mouse test exists (Humby et al.,2013), but is currently not widely used. One reason is the labor intensive training duration (~44 days).
To overcome this drawback, we developed an automated stop signal task by integrating an RFID-based sorting system with an operant box. The sorting system allows mice to enter the operant box in a self-motivated manner throughout the day without interference from the experimenter. With this setup we were able to reduce the training duration to a maximum of 26 days while also reducing daily labor for the experimenter. As expected our data show a decrease in behavioural inhibition following an increase in the time delay between go and stop signal which is a hallmark of the stop signal task.

Methods

Six RFID chip implanted female mice (C57Bl/6JRj; 3 month) were trained on the automated stop signal task to analyse the effect of different time delays between go and stop signal on the probability of behavioural inhibition.

Sorting Procedure:

- Mouse is recognized at Rd1, door 1 opens
- Mouse enters sorter, door 1 closes
- 30s verification interval: Rd 2 and 3 verify that only a single animal has entered
- Door 2 opens and mouse enters operant box

Behavioral Procedure:

For the stop signal task mice learn stepwise to respond to the left receptacle (trial initiation which is go signal) before poking into the middle receptacle (go response) within a short limited hold period. On stop trials a stop signal occurs following trial initiation upon which the animal has to withhold its go response. Failure to do so results in 45s timeout and an air puff. Rewards are collected from the right receptacle following successful trials.

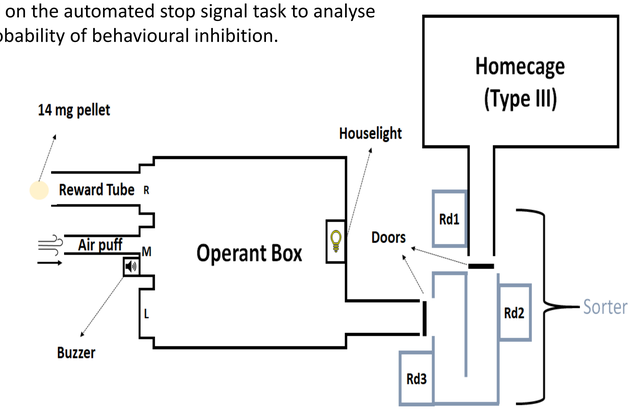

Fig 1. Experimental Setup; Rd: ID reader (PhenoSys); R M L: receptacle (right, middle and left) each equipped with LED light and nose poke detector. Buzzer: 70 dB,4.5 kHz as stop signal. Houselight: Signals timeout.

Stage	Aim	Limited hold (LH)	Waiting duration after stop signal	Stop signal (70 dB,4.5 kHz)	Behavioural schema
Habituation to sorter and operant box	To teach the place of reward and get habituate to sorting process	-	-	-	
Initiation response training (poke to left)	To teach initiation response	-	-	-	
Go response Training (poke to middle)	To teach go response and get fast go response	From 30 s to 2.1s	-	-	
Stop Signal Training	To teach stopping upon hearing stop signal (stopping in stop trials>%80)	2.1-2.4 s (individually adjusted to ensure >%70 completed go trials)	From 400 ms to limited hold (LH) duration	duration:300 ms to 150 ms, coincides with initiation response (go signal), %20 of trials	
Probe trials with delays	To observe the effect of the time delays on probability of behavioral inhibition.	2.1-2.4 s	LH duration	Duration:150 ms, with time delays relative to mean reaction time Delay:100 ms,300 ms and 500 ms before mean reaction time	

Data analysis:

The probability of inhibition is calculated for each delay and corrected for the probability of omission in go trials according to Tannock et al. 1989. Corrected inhibition probabilities are then converted to odds and a logistic regression model is fitted to data by taking delays as the fixed effect and individual as the random effect.

Results

Baseline performance from zero-delay sessions:

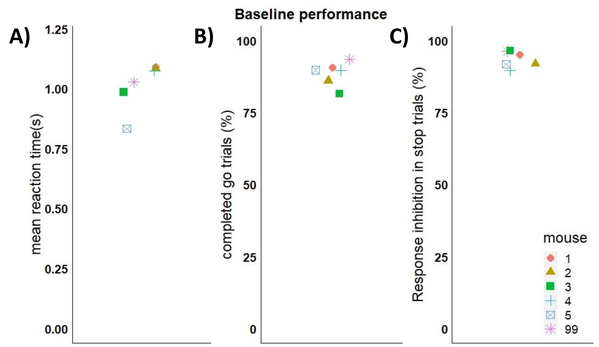

Fig 2. Baseline performance from last day of stop signal training (without delay). A) mean reaction times of animals . B) percentage of completed go trials C) percentage of behavioral inhibition in stop trials.

- **All animals animals responded fast (1.09-0.83 s) and reliably (>70% completed go trials) and they were also able to reliably stop upon hearing the stop signal (% 80 stopping in stop trials) in the zero-delay sessions.**

Inhibition curve with the predicted values from the model:

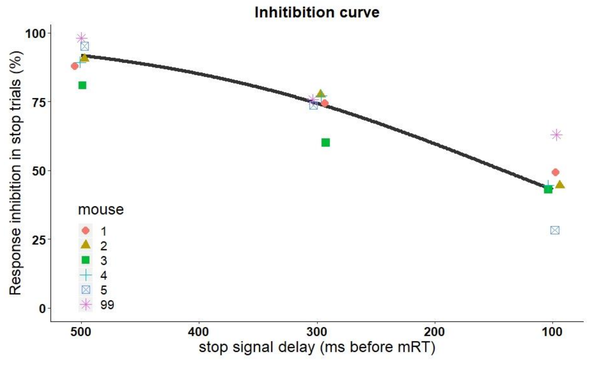

Fig 3. Probability of behavioral inhibition across time delays. Black curve is fitted for the predicted behavioral inhibition from the logistic regression model.

- **Probability of response inhibition is decreased with increasing stop signal delay which is predicted by our model. Our model also produces an S-shaped curve which is consistent with the theoretical framework from the horse race model.**
- **For more info about the theoretical framework and its simulation :**

https://github.com/karengu30/ShinyApp-Two-Horse-Race-Model

Logistic regression model-effect of stop signal delay

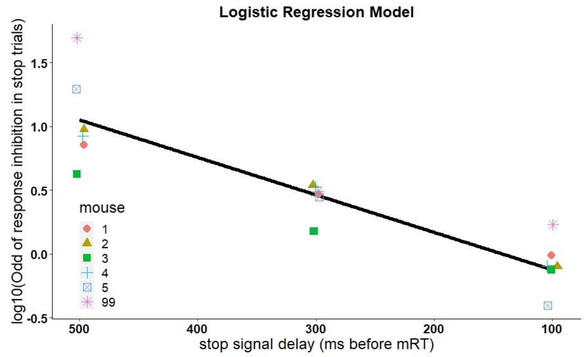

Fig 4. Log_{10} of odds of behavioral inhibition across the time delays. Black line is fitted for the main effect (delay) for the model. With increasing time delay odds of behavioral inhibition decreases.

- **Log_{10} of odds of response inhibition in stop signal trials was decreasing with increasing stop signal delay (95% CI: [-2.90 * 10^3, -2.86* 10^3]).**

Conclusion

- Our setup allows studying action cancellation in an automated and high-throughput fashion which leads to less laborious and faster experiments. With our system all mice proceeded to probe trials within 26 days of training while with the traditional method it takes ~44 days of training to proceed to probe trials.
- In the task the probability of inhibiting the action is decreased with increasing stop signal delay, which is a hallmark of the stop signal task.
- In the future, our setup will allow investigating the underlying biology of action cancellation with the stop signal task in a more efficient way. This improvement has the potential to increase the use of this highly translational behavioural task in rodent studies.

Acknowledgments

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References

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